THE CHOLINE AND PYRIDOXINE CONTENT OF MEATS [1]

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(Received for publication June 1, 1944)

Although choline and pyridoxine are recognized as essential factors for normal animal metabolism there is a paucity of information concerning the distribution of these substances even in the main dietary constituents. Since chemical and microbiological methods for the determination of choline (Engel, '42) and pyridoxine (Atkin et al., '43) were available, we have used these methods to determine the choline and pyridoxine content of a number of fresh, cooked and commercially prepared meats.

EXPERIMENTAL

The methods of preparing the samples have been reported previously (McIntire et al., '43), (Schweigert et al., '44) and (McIntire et al, '44). All determinations were made on undried fresh and cooked meats. Values reported on the dry basis were calculated from results of the determinations on fresh meats.

The method used for the determination of choline was essentially that reported by Engel ('42). Samples of fresh and cooked meats were minced finely and extracted over night with boiling methanol. The alcohol extracts were evaporated to dryness on a steam bath, and the residues were saponified for 2 hours with boiling aqueous barium hydroxide. After saponification the barium soaps were filtered off, and the choline was precipitated as choline reineckate by adding 5 ml. of an alcoholic solution of 2% ammonium reineckate to the filtrate. Complete precipitation of the choline reineckate was assured by allowing the mixture to stand in a —4°C. cold room for 12 hours. The bright red choline reineckate precipitate was then washed with 2–5 ml. portions of cold ethanol and water until the washings were free of color. The choline reineckate was dissolved in approximately 15 ml. of acetone and the concentration determined by measuring the density

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

Supported in part by a grant from the National Live Stock and Meat Board.

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of the color in an Evelyn colorimeter. This method proved very satisfactory and duplicate determinations with few exceptions checked within 5%.

A modification of the yeast method of Atkin et al. ('43) was used for the determination of pyridoxine. Yeast cultures were grown in 125 ml. Erlenmeyer flasks in a 30°C. incubator without shaking for 19 hours. Maximum liberation of the pyridoxine was attained when the meat samples were autoclaved for 1 hour at 20 lbs. pressure in 180 ml. of 0.1 N HCl. Recoveries varying from 83 to 101% were obtained under these conditions. Considerable variation was observed in duplicate analyses although all values recorded in this paper were checked within 10%. The choline and pyridoxine contents of veal, lamb, pork, and beef as well as several special organs and commercially prepared meats were determined (table 1).

DISCUSSION

Choline values for the various meats agree with the available values reported by Engel ('43), who has made a general survey of the choline content of animal and plant products. Our values for roast veal were 133 to 144 mg., for lamb chops 76 mg., and for ham 101 to 129 mg. of choline per hundred grams of meat compared to 113 mg. for veal roast, 106 mg. for lamb chops, and 88 mg. per hundred grams for ham reported by Engel. Our choline values for kidney and liver, 240 to 280 mg. and 470 to 570 mg. per hundred grams compare with 330 and 486 to 708 mg. of choline per hundred grams of kidney and liver reported by Engel.

Most pyridoxine values obtained in this work are in good agreement with those obtained by Henderson et al. ('41) with the rat assay method, although some of our values for the muscle meats are slightly lower. These discrepancies are attributed to the fact that fat was trimmed from the meats assayed by Henderson et al. They reported 0.3 mg. of pyridoxine for lamb muscle, 0.59 mg. for ham, and 0.4 mg. for veal per hundred grams. The range of our values for these meats are 0.22 to 0.33 mg. for lamb, 0.25 to 0.37 mg. for ham and 0.25 to 0.45 mg./100 gm. for veal. The values for organ meats are in good agreement with the values reported by Henderson et al. ('41) which are 0.44 mg. of pyridoxine for beef kidney, 0.24 mg. for beef heart, 0.73 mg. for beef liver and 0.12 mg./100 gm. for beef tongue. Our corresponding values for these meats were 0.36 to 0.42 mg. for beef kidney, 0.29 mg. for veal heart, 0.5 to 0.99 mg. for beef liver and 0.13 mg./100 gm. for beef tongue.

Vitamin retention studies were carried out after cooking veal and lamb and curing ham. The method for calculating retentions has been previously described (McIntire et al. '43a). The veal samples were roasted, braised and stewed and the lamb samples were roasted, broiled, and stewed.

The destruction or leaching of choline from the meats during any of the cooking or curing processes is negligible since 87 to 114% of the choline was retained in the meat.

TABLE 1

Choline and pyridoxine content of meats. (mg./100 gm.)

SAMPLE	CHOLINE				PYRIDOXINE			
	Fresh		Dry		Fresh		Dry	
	Range	Avg.	Range	Avg.	Range	Avg.	Range	Avg.
Veal								
Leg	95–108	102	366–432	389	.34–.41	0.37	1.3 –1.6	1.4
Roast leg	125–141	132	338–392	360	.20–.21	0.20	.50– .57	.53
Shoulder	83–100	93	268–373	337	.25–.38	.30	.81–1.4	1.1
Roast shoulder	133–143	139	310–376	343	.12–.15	.14	.30– .37	.33
Sirloin chop	87–105	96	242–404	317	.36–.45	.41	1.0 –1.7	1.4
Braised chop	128–157	140	242–342	285	.10–.12	.11	.21– .24	.23
Shoulder chop	92–101	97	307–422	376	.32–.38	.35	1.1 –1.4	1.2
Braised chop	149–156	154	317–400	366	.11–.14	.12	.22– .34	.28
Stew meat	94–100	96	336–400	367	.32–.34	.33	1.3	1.3
Cooked stew	137–149	142	360–378	370	.09–.10	.10	.24– .27	.26
Lamb								
Leg	75– 92	84	262–317	290	.24–.33	.29	.80–1.1	.93
Roast	122–124	123	284–295	290	.10–.13	.12	.23– .31	.27
Sirloin chop	75– 77	76	179–198	189	.21–.22	.22	.50– .56	.53
Broiled chop	100–126	113	204–252	228	.08–.13	.11	.17– .26	.22
Stew meat	76– 82	79	222–230	226	.22–.23	.23	.62– .66	.64
Cooked stew	116–128	122	247–291	269	.05–.06	.06	.11– .14	.13
Pork								
Ham	101–129	120			.25–.37	.33		
Cured ham	98–129	122			.15–.21	.19		
Beef								
Liver	470–570	510			.50–.99	.71		
Round	65– 70	68			.31–.43	.37		
Tongue	108	108			.13	.13		
Heart	170	170			.29	.29		
Braised heart	200–275	238						
Kidney	240–284	262			.36–.42	.39		
Brain	399–420	410			.16	.16		
Miscellaneous								
Bologna	60	60						
Frankfurters	57	57			.13	.13		
Pork links	48	48						
Canadian bacon	80	80						

A low pyridoxine retention in meat after cooking was quite surprising in view of the fact that this vitamin is considered to be one of the heat stable members of the B complex. Retentions of pyridoxine in the meat after various cooking methods ranged from 14 to 42% and retention in ham after curing averaged 57%. A few of the drippings from the cooked meat were available for analysis, but all were found to contain less than 6 % of the total vitamin in the meat. A summary of the pyridoxine retention studies is shown in table 2.

TABLE 2

Retention of pyridoxine in meat. (All values in %.)

VEAL			LAMB			HAM
Roasting	Braising	Stewing	Roasting	Broiling	Stewing	Curing
41	19	18	28	40	18	53
36	22	18	29	28	14	59
42	17					58
26	22					58
31	15					
29	20					
Avg. 34	19	18	28.5	34	16	57

A few correlations between the type of cooking and the pyridoxine retention are apparent. Roasting and broiling resulted in higher retention than stewing and braising. The average retention after roasting veal was 34% and after roasting lamb 28%. After broiling lamb the average amount of pyridoxine retained was 34%. The retention after stewing veal and lamb was 18 and 16%, respectively, while retention after braising veal was 18%.

Similar correlations were found for thiamine retention in veal and lamb after these cooking processes (McIntire et al., '43). It was suggested at that time that the extracting action on the meat of the condensing vapors and surrounding liquid during the braising and stewing processes was responsible for the greater thiamine losses from the meat. The pyridoxine retentions after these cooking processes can also be explained on this basis.

SUMMARY

A survey of the choline and pyridoxine content of veal, lamb, pork, beef, special organ meats, and commercially prepared meats has been made. Muscle meats contain from 0.22 to 0.45 mg. of pyridoxine and 70 to 144 mg. of choline per hundred grams. Kidney, heart and liver are somewhat higher.

The vitamin retention after curing and cooking was studied. Between 87 and 114% choline retention was observed while the pyridoxine retention ranged from 14 to 42% after cooking. An average of 57% of the pyridoxine was retained in ham after curing.

LITERATURE CITED

ATKIN, L., A. S. SCHULTZ, W. L. WILLIAMS AND C. N. FREY 1943 Yeast microbiological methods for determination of vitamins (pyridoxine). Ind. Eng. Chem. Anal. Ed., vol. 15, p. 141

ENGEL, R. W. 1942 Modified methods for the chemical and biological determination of choline. J. Biol. Chem., vol. 144, p. 701.

———— 1943 The choline content of animal and plant products. J. Nutrition, vol. 25, p. 441.

HENDERSON, L. M., H. A. WAISMAN AND C. A. ELVEHJEM 1941 The distribution of pyridoxine in meat and meat products. J. Nutrition, vol. 21, p. 589.

MCINTIRE, J. M., B. S. SCHWEIGERT AND C. A. ELVEHJEM 1943 The retention of vitamins in veal and lamb during cooking. J. Nutrition, vol. 26, p. 621.

MCINTIRE, J. M., B. S. SCHWEIGERT, L. M. HENDERSON AND C. A. ELVEHJEM 1943a The retention of vitamins in meat during cooking. J. Nutrition vol. 25, p. 143.

MCINTIRE, J. M., B. S. SCHWEIGERT, E. J. HERBST AND C. A. ELVEHJEM 1944 Vitamin content of variety meats. J. Nutrition, vol. 28, p. 35.

SCHWEIGERT, B. S., J. M. MCINTIRE AND C. A. ELVEHJEM 1944 The retention of vitamins in pork hams during curing. J. Nutrition, vol. 27, p. 419.

INADEQUACY OF LACTOSE AND BETA-LACTOSE AS DIETARY CARBOHYDRATES FOR THE RAT

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(Received for publication May 8, 1944)

A strain difference in the development of cataract has been reported in the rat both in relation to the time of onset and to the degree of incidence following lactose feeding (Mitchell, '36). Further studies have demonstrated that the cataractogenic properties of these rations were not due to lactose per se but rather to galactose liberated from this sugar on hydrolysis (Mitchell, '35; Day, '36). In the present communication we wish to report the inadequacy of lactose and beta-lactose [1] as the sole dietary carbohydrate in purified rations for the rat, an inadequacy varying both in extent and degree with the strain and not shared by galactose, glucose, sucrose or corn starch when one of the latter replaces lactose or beta-lactose in the ration.

PROCEDURE AND RESULTS

Two strains of rats were employed in the present experiment: (1) animals of the Long-Evans strain (both colored and albino); and (2) animals of the U.S.C. strain [2] (albino). At weaning (21–23 days) litter mates of both sexes were placed on eight different dietary regimes (table 1). Seven groups of rats were maintained on diets differing solely in composition of dietary carbohydrate and an eighth group (H) was maintained on Purina dog chow which was supplemented once a week with lettuce. A total of ninety-four Long-Evans and sixty-four U.S.C. rats were employed in the following experiment.

After 72 hours on the feeding regimes, Long-Evans rats on the beta-lactose ration differed markedly from litter mates on control diets or animals of the U.S.C. strain on the same diet. Ingestion of the beta-lactose ration resulted in a syndrome consisting of severe diarrhea,

[1] Beta-lactose or anhydrous lactose is formed by crystallizing lactose at a temperature of 93.5°C. It is a white crystalline powder, sweeter than lactose, and about twice as soluble as the latter in water. In the presence of water at 75°C. it gradually reverts to ordinary lactose.

[2] A modified Wistar strain.

unthrifty appearance, ruffled fur, edema of the hind paws, various degrees of alopecia, and finally death. Length of survival averaged 4.9 days (2–17). Symptoms varied considerably from animal to animal. Approximately 50% of the rats exhibited varying degrees of alopecia at the time of death, this being most pronounced in animals surviving longest on the ration. Loss of hair was observed in some animals as

TABLE 1

Composition of experimental diets and distribution of rats.

DIETARY COMPONENTS	DIET							
	A	B	C	D	E	F	G	H
Lactose [1]	73.2							
Beta-lactose [2]		73.2					36.6	
Galactose [2]			73.2					
Glucose				73.2				
Sucrose					73.2		36.6	
Corn starch						73.2		
Vitamin test casein [3]	22.0	22.0	22.0	22.0	22.0	22.0	22.0	
l (—) Cystine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	
Salt mixture [4]	4.5	4.5	4.5	4.5	4.5	4.5	4.5	
Distribution of rats								
Strain: Long-Evans	10	42	4	6	10	6	6	10
U.S.C.	12	12	2	6	10	6	6	10

To each kg. of the above mixture were added the following synthetic vitamins: thiamine hydrochloride 20 mg., riboflavin 20 mg., pyridoxine hydrochloride 20 mg., calcium pantothenate 100 mg., nicotinic acid 100 mg., p-aminobenzoic acid 200 mg., inositol 500 mg., choline chloride 1200 mg., and 2-methyl naphthoquinone 5 mg. In addition each rat received the following daily supplement: corn oil (Mazola) 800 mg., alpha-tocopherol 0.5 mg., and a vitamin A and D concentrate [5] containing 50 U.S.P. units of vitamin A and 5 U.S.P. units of vitamin D. All rations were fed ad libitum.

[1] U.S.P. Lactose, Braun Corporation, Los Angeles, California.
[2] National Milk Sugar Company, New York, New York.
[3] S.M.A. Corporation, Chagrin Falls, Ohio.
[4] Salt mixture no. 1 (Sure, '41).
[5] Nopco Fish Oil Concentrate, assaying 800,000 U.S.P. units vitamin A and 80,000 U.S.P. units vitamin D per gram.

early as 48 hours after administration of the diet. Alopecia was particularly marked between the ears and on the dorsal surface of the neck. Other areas occurred in the lumbar region, the belly, and the area immediately overlying the vertebral column. Other animals died with no evidence of alopecia, but with marked swelling of the hind paws, an unthrifty appearance, and ruffled fur frequently caked with fecal material. Eight to 12 hours before death a marked reduction oc-

curred in body temperature, animals became weak and listless, body tone poor, and when animals were placed on their back righting was markedly delayed.

Rats of the Long-Evans strain receiving the lactose diet differed in several respects from those on the beta-lactose ration. Length of survival was prolonged, averaging 11.5 days (6–21) in contrast to the 4.9 days of the beta-lactose series. Alopecia was not observed before the eighth day of feeding and in general was less pronounced. Diarrhea developed more slowly and was less extensive; edema of the hind paws was not observed; but in general the syndrome paralleled that of the beta-lactose series. At autopsy the only gross abnormality observed was a marked dilatation of the cecum. Rats failed to grow satisfactorily on the lactose and beta-lactose rations and gained less than 7 gm. a week in contrast to 20 ± 2 gm. for litter mates receiving any of the other diets except galactose. Preceding death a drop in body weight occurred which approximated 20% of the maximum weight. Animals on the galactose ration differed, however, from litter mates on glucose, sucrose or corn starch rations; their growth was poor, the animals actually losing 10% of their body weight during the first week on the ration. During the second week weight loss was recovered so that by the fourteenth day animals had attained weaning weight. Subsequent to this period growth averaged 10 gm. a week for the next 2 weeks at which time feeding was discontinued. Mature cataracts developed 22 to 24 days after the start of the feeding regime in contrast to the 11-day period preceding development in the U.S.C. strain (two rats only). Food consumption was increased averaging 17.5% of body weight per day as contrasted with 10.5% in litter mates receiving glucose, sucrose or corn starch rations, and water consumption was similarly elevated averaging 118% of body weight per day (63–215%) in contrast to a daily consumption of 12 ± 1% in litter mates receiving glucose, sucrose or corn starch rations for a similar period.

A difference was observed between the Long-Evans and the U.S.C. strains as a result of ingesting lactose or beta-lactose rations. Length of survival was prolonged in the U.S.C. strain, averaging 22 days (16–28) for rats on the beta-lactose diet and 26 days (19–28) for the lactose ration contrasting with 4.9 and 11.5 days respectively for animals of the Long-Evans strain. That these differences are significant is evident from the fact that the longest survival time noted for Long-Evans rats on diets A or B was less than the averages observed for U.S.C. rats on similar rations. In the U.S.C. strain, for the first 10 days of feeding, animals on the lactose or beta-lactose diet were indistinguish-

able from litter mates on control rations, with the exception of retarded growth. Subsequent to this period, symptoms similar to those in the Long-Evans strain developed. In general, alopecia was not as marked as in the latter strain; only ¼ of the rats exhibited thinning or loss of fur with the affected area primarily the lower back and abdomen. At autopsy the only gross abnormality observed was a marked dilation of the cecum [3] approaching twenty times its extent in litter mate controls. No significant difference was observed between the two strains after feeding the other diets.

Effects of previous diet on beta-lactose feeding

Ten Long-Evans rats were placed at weaning on rations E and H (five animals per group). Both diets were fed ad libitum. After 28 days of feeding rats were transferred to a beta-lactose ration (diet B). No significant difference in response was noted between the two groups. Survival averaged 14 ± 3 days. By the seventh day alopecia was marked in both series, animals developed acute diarrhea, lost weight rapidly, and at autopsy averaged 30% less than when placed on the beta-lactose ration. There is some evidence that the age at which rats were started on the beta-lactose ration affected response to feeding. When rats were placed on a beta-lactose ration at weaning, survival averaged 4.9 days; when placed on the ration at 49 days of age, survival averaged 14 days; when started at 150 days of age (three rats) animals remained free of symptoms for 28 days at which time feeding was discontinued.

Effects of previous maternal diet on lactose and beta-lactose feeding of young

The experimental animals of the following series consisted of young born to mothers of the U.S.C. strain that (1) had been raised from weaning on a stock diet and bred on the same ration (Deuel et al., '33), (2) raised from weaning on a stock diet but changed to a Sherman diet 2 months before mating (I), and (3) raised from weaning and bred on a Sherman diet for nine generations (II). At weaning the young were placed on rations A and B which were fed ad libitum.

No significant difference was observed at autopsy in animals dying on lactose or beta-lactose rations regardless of the previous diet of the parents. With the exception of young born on a stock diet, how-

[3] We wish to express our sincere appreciation to Professor E. M. Hall, Department of Pathology, University of Southern California Medical School, for his help at autopsy.

ever, a certain percentage of rats placed on lactose or beta-lactose rations survived 8 weeks of feeding at which time the feeding was discontinued. Subsequent to the third week a marked improvement occurred in those rats which did survive, the animals gained weight, new hair replaced the areas of alopecia, and diarrhea improved.

TABLE 2

The effect of maternal diet on survival after transferring weaned rats to lactose or beta-lactose diets.

PREVIOUS MATERNAL DIET	NUMBER OF ANIMALS	PERCENTAGE OF ANIMALS DYING	AVERAGE SURVIVAL TIME OF DECEDENTS [1]	PERCENTAGE OF ANIMALS SURVIVING
Lactose tests (A)				
Stock diet	12	100	25.8 ± 0.7	0
Sherman I	10	70	17.1 ± 1.8	30
Sherman II	10	60	24.7 ± 0.9	40
Beta-lactose tests (B)				
Stock diet	12	100	21.9 ± 0.9	0
Sherman I	10	80	16.1 ± 2.2	20
Sherman II	17	47	21.2 ± 1.0	53

[1] Including standard error of the mean calculated as follows: $\sqrt{\frac{\Sigma d^2}{n}} / \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

Failure of survival on lactose or beta-lactose rations in rats born on a stock diet as compared with the control series appears to be significant, but the number of animals in each group is too small to permit a final statement on this matter. Of animals that failed to survive these rations no protective action on length of survival was noted that could be attributed to the previous maternal diet.

Effect of dietary supplements on beta-lactose feeding

The recent report by Boutwell et al. ('43, '44), that butter fat is superior to corn oil in promoting growth of rats maintained on purified rations containing lactose as the sole carbohydrate, suggested a possible interrelationship between the beta-lactose syndrome and dietary fat. Accordingly, corn oil was omitted from the ration and butter fat, oleomargarine, and lard incorporated to the extent of 10% of the diet replacing a similar quantity of beta-lactose. Groups of four rats each of the Long-Evans strain were placed on the above diets at weaning and fed the ration ad libitum. Animals receiving the lard supplement survived an average of 2.4 days, those receiving oleomargarine 3 days, and those on the butter fat supplement 3.2 days. Butter fat, oleo-

margarine and lard were ineffective, at least at the level used, in preventing the beta-lactose syndrome in the Long-Evans rat.

Incorporation of dried mammalian liver [4] to the extent of 10% of the ration failed to maintain life, but prolonged slightly the survival on the ration (7 ± 2 days). Similarly, dried tuna liver [5] increased the survival time somewhat more (11 ± 3 days). In contrast to animals on other supplements, rats receiving liver-containing rations gained weight for the first 5 to 7 days on the ration paralleling the growth of animals on glucose- or sucrose-containing diets; subsequent to this period they lost weight and exhibited a typical beta-lactose response.

Wheat germ oil,[6] cream, biotin,[7] and brewer's yeast were similarly ineffective. Length of survival on a ration containing wheat germ oil (800 mg. daily) in place of corn oil averaged 3.2 days, on a cream supplement (800 mg. daily) 6 days, while the oral administration of 5 μg. of biotin per day failed to prolong survival beyond the fifth day. Incorporation of brewer's yeast to the extent of 10% of the ration with all crystalline vitamins omitted was equally ineffective.

Attempts to rule out diarrhea as a contributory cause of death were unsuccessful. Citrus pectin [8] and apple powder [9] were incorporated into purified rations containing beta-lactose as the sole carbohydrate. Long-Evans rats in groups of five were placed at weaning on the above diets, containing pectin (5 and 10%), and apple powder (10%), and the rations fed ad libitum. Diarrhea was not controlled, and animals died as rapidly as did the unsupplemented controls.

DISCUSSION

Although there have been a number of reports in the literature where growth retardation, diarrhea, and cataract formation followed the administration of lactose (Whittier et al., '35; Mitchell and Dodge, '35; Day, '36; and Mitchell, '36), this is the first report (with the possible exception of Nielsen and Black, '44) where the administration of this disaccharide resulted fatally. In this study beta-lactose apparently possessed a more pronounced effect than lactose, a difference which was not noted by Mitchell and Dodge ('35). These discrepancies may in part be due to strain differences, the composition of the non-carbo-

[4] Dried liver from Wilson Laboratories, Chicago, Illinois.

[5] Kindly furnished by Dr. Sven Lassen, Van Camp Sea Food Company, Terminal Island, California.

[6] Viobin Corporation, Monticello, Illinois.

[7] Biotin concentrate no. 200, S.M.A. Corporation, Chagrin Falls, Ohio.

[8] N. F. Pectin, California Fruit Growers Exchange, Ontario, California.

[9] Apella Corporation, Selah, Washington.

hydrate portion of the diet, the previous dietary history of the parents or such environmental conditions as temperature and humidity.

Whether strain differences alone could account for the above discrepancies is problematical although there is considerable evidence to indicate that rats of different strains may respond differently to the same dietary.[10] Long-Evans rats on a beta-lactose ration developed acute diarrhea within 24 hours of the initial feeding; U.S.C. rats on the same ration remained free of diarrhea for at least 8 days. Long-Evans rats on a beta-lactose ration survived an average of 4.9 days with 100% mortality; U.S.C. rats on the same ration survived 20 days with 28% showing spontaneous recovery and freedom from symptoms. Fifty per cent of the Long-Evans rats on a beta-lactose ration developed alopecia; U.S.C. rats on the same diet developed this condition in only half this number. Similar discrepancies were observed in respect to lactose feeding.

The pathologies observed on lactose and beta-lactose rations suggested the possibility of alterations in the intestinal flora acting as a contributory factor in these conditions. It has long been known that lactose feeding brings about changes in the pH of the contents of the whole intestinal tract below the duodenum (Hudson and Parr, '24; Robinson and Duncan, '31) and favors the development of an acidophilic flora (Rettger and Cheplin, '23; Hudson and Parr, '24). Such changes have not been observed in diets containing glucose, sucrose, galactose or corn starch in place of the lactose. Inasmuch as microorganisms present in the intestinal tract are known to produce biotin, folic acid, inositol, and other members of the vitamin B complex in addition to vitamin K, the possibility of alterations of the intestinal flora from the normally proteolytic to the acidophilic type precipitating nutritional deficiencies either through failure of intestinal synthesis or through increased utilization of factors already present is worthy of further consideration. Boutwell et al. ('43) and Gyer et al. ('43) have suggested essentially the same possibilities. An analysis of earlier diets utilizing lactose or beta-lactose as the sole dietary carbohydrate has revealed the fact that in almost every case natural sources of the vitamin B complex were employed in addition to unextracted casein and fats such as lard or Crisco. The possibility of nutritional factors being present in these materials in sufficient concentration to make good any deficiency resulting from alterations in the intestinal flora has not been excluded.

[10] Experiments are now in progress with the Sprague-Dawley rat. Preliminary results indicate that the development of diarrhea in this strain on diets A and B is similar in extent and time of appearance to that observed in the U.S.C. strain.

The findings of Boutwell at al. ('43, '44) and Geyer et al. ('43) that butter fat and lard are superior to corn oil in promoting growth of rats receiving lactose as the sole carbohydrate would seem to indicate this very condition although Deuel et al. ('44) have noted no differences in nutritive values of butter fat and various vegetable fats when fed with skimmed milk powder. Geyer et al. ('43) have noted that rats maintained on a purified ration containing 48% lactose and 28% corn oil developed "rough and discolored fur coats, blood-stained noses and scaly paws (when the humidity was not abnormally high)." In their most recent report, Boutwell et al. ('44) have reported the occurrence of alopecia on a similar ration. Nielsen and Black ('44) employing diets containing 73% lactose and 5% corn oil reported kidney damage and many early deaths.

That alterations of the intestinal flora are not the sole or even the predominant factors involved in precipitating the "beta-lactose syndrome" might be indicated, however, by the failure of dietary supplements to modify the response to beta-lactose feeding in the Long-Evans rat. Butter fat, oleomargarine, lard, wheat germ oil, cream, biotin, and brewer's yeast were all ineffective in promoting survival or prolonging the appearance of symptoms. Of all the substances tested only liver gave some indication of a protective effect. Furthermore, the rapidity with which symptoms developed suggested that some factor other than altered intestinal synthesis is involved. Mitchell and coworkers ('39) have pointed out that the presence of the gluconate radical either as the sodium or calcium salt inhibits utilization of lactose by the rat. According to these workers the gluconate ion inhibited lactase activity in the intestinal tract resulting in definite toxic symptoms, retarded growth, poor survival, moderate to severe diarrhea and lowered blood sugar. In the present study data on blood sugar and lactase activity of the intestinal tract are lacking, but further studies on the effects of substances either of dietary or intestinal origin inhibiting lactase activity might provide information on the mechanism involved in the beta-lactose syndrome.

Variations in lactase activity might also be a factor in explaining strain differences. There is evidence to indicate that the diarrhea resulting from ingestion of lactose or beta-lactose is related to failure of enzymatic hydrolysis and the excess of lactose remaining as such in the alimentary tract. Failure of pectin and apple powder to counteract the diarrhea resulting from ingestion of beta-lactose rations would be consistent with this hypothesis. Inasmuch as the Long-Evans rat developed acute diarrhea within 24 hours after ingesting a beta-lactose

ration, contrasting with 8 and 10 days necessary for this condition to develop in the U.S.C. rat, differences in lactase activity of the two breeds might be expected. Further variations in lactase activity might be expected in U.S.C. rats exhibiting spontaneous recovery on a lactose or beta-lactose ration as contrasted with litter mates dying on the same diet.

SUMMARY

Male and female rats of the Long-Evans and U.S.C. strains were placed at weaning on purified rations containing lactose, beta-lactose, glucose, galactose, sucrose and corn starch as the sole source of carbohydrate. The following observations were made: (1) rats failed to survive on purified rations containing lactose or beta-lactose as the sole carbohydrate; (2) rats developed alopecia when fed the above lactose or beta-lactose rations; (3) a strain difference was observed in the incidence and degree of alopecia as well as the length of survival of rats fed lactose or beta-lactose rations; (4) rats fed a beta-lactose ration died sooner and developed more severe alopecia than those fed a lactose diet; (5) length of survival was correlated with severity of diarrhea; and (6) a relationship was observed between degree of mortality and previous maternal diet.

LITERATURE CITED

BOUTWELL, R. K., R. P. GEYER, C. A. ELVEHJEM AND E. B. HART 1943 Further studies on the growth-promoting value of butter fat. J. Dairy Science, vol. 26, p. 429.

———— 1944 Effect of flavor on the nutritive value of fats. Proc. Soc. Exp. Biol. Med., vol. 55, p. 153.

DAY, P. L. 1936 Blood sugar in rats rendered cataractous by dietary procedures. J. Nutrition, vol. 12, p. 395.

DEUEL, H. J., JR., E. M. MACKAY, P. W. JEWEL, M. GULICK AND C. F. GRUNEWALD 1933 Studies on Ketosis. II. The comparative glycogen formation and retention after the administration of glucose, galactose and lactose. J. Biol. Chem., vol. 101, p. 301.

DEUEL, H. J., JR., E. MOVITT, L. F. HALLMAN AND F. H. MATTSON 1944 Studies on the comparative nutritive value of fats. I. Growth rate and efficiency of conversion of various diets to tissue. J. Nutrition, vol. 27, p. 107.

GEYER, R. P., R. K BOUTWELL, C. A. ELVEHJEM AND E. B. HART 1943 Rations for the study of the relative nutritive value of fats and oils. Science, vol. 98, p. 499.

HUDSON, N. P., AND L. W. PARR 1924 Relation of reaction of intestinal contents to diet and flora. J. Infect. Dis., vol. 34, p. 621.

MITCHELL, H. S. 1935 Cataract in rats fed on galactose. Proc. Soc. Exp. Biol. and Med., vol. 32, p. 971.

———— 1936 Susceptibility of different strains of rats to nutritional cataract. J. Nutrition, vol. 12, p. 447.

MITCHELL, H. S., G. M. COOK AND K. L. O'BRIEN 1939 Effect of several calcium salts on the utilization of lactose. J. Nutrition, vol. 18, p. 319.

MITCHELL, H. S., AND W. M. DODGE 1935 Cataract in rats fed on high lactose rations. J. Nutrition, vol. 9, p. 37.

NIELSEN, E., AND A. BLACK 1944 Role of inositol in alopecia of rats fed sulfasuxidine. Proc. Soc. Exp. Biol. and Med., vol. 55, p. 14.

RETTGER, L. F., AND H. A. CHEPLIN 1923 A treatise on the transformation of the intestinal flora with special reference to the implantation of Bacillus acidophilus. Yale University Press, New Haven.

ROBINSON, C. S., AND C. W. DUNCAN 1931 The effect of lactose and the acid-base value of the diet on the hydrogen ion concentration of the intestinal contents of the rat and their possible influence on calcium absorption. J. Biol. Chem., vol. 92, p. 435.

SURE, B. 1941 Dietary requirements for fertility and lactation. XXIX. The existence of a new dietary factor essential for lactation. J. Nutrition, vol. 22, p. 449.

WHITTIER, E. O., C. A. CARY AND N. R. ELLIS 1935 The effects of lactose on growth and longevity. J. Nutrition, vol. 9, p. 521.

THIAMINE CONTENT OF HEN EGGS DURING INCUBATION [1]

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(Received for publication June 9, 1944)

Relatively few studies have been made on the vitamin content of developing eggs. Suomalainen ('39 a) found that the vitamin A content decreased while vitamin C was synthesized (Suomalainen, '39 b). Snell and Quarles ('41) followed the concentration of several of the vitamins of the B complex in hen eggs during incubation. They reported that nicotinic acid and inositol were synthesized, but the total amount of pantothenic acid, riboflavin and biotin remained essentially unchanged. Woolley ('42) reported that the total content of inositol in the hen egg does not increase during incubation but the proportion of the total amount which is free and extractable by water does.

Williams, Taylor and Cheldelin ('41) have observed the B vitamin content of rat and chick organs at several different ages. Marked differences were found in the thiamine content of the various organs of the 15-day embryo when compared with those of the young chick. The present study was undertaken to determine the effect of incubation on the thiamine content of the egg. In order to do this, the entire content of the egg at various stages of development was analyzed.

While this study was in progress, the work of Westenbrink and Van Leer ('41) became available in abstract form. They approached the problem by studying separately the drop in the total thiamine in the yolk and the increase in thiamine content of the developing embryo. They concluded that a considerable part of the thiamine originally in the egg fails to appear in the embryo and is, therefore, apparently lost during development.

In our assays of the total thiamine content of the egg we have found no significant change in either fertilized or sterile eggs during the 3 weeks at 37–38°C. required for normal development.

[1] Aided by a Research Fellowship from Swift and Company, Chicago, Illinois.

EXPERIMENTAL

The eggs from White Leghorn hens were supplied by Roger's Farms, a commercial hatchery. The eggs were weighed and placed in a Waring Blendor. The weights of the empty shells with their shell membranes were also recorded. After blending, 40 gm. were suspended in 250 ml. to give a convenient dilution for our assay procedure. At first solutions were made acid to litmus and autoclaved for 15 minutes at 15 pounds (Schultz, Atkin and Frey, '37). This resulted in some destruction of thiamine in the samples. Eventually it was found best to adjust the sample to pH 5.00 and autoclave for 10 minutes at 10 pounds. Samples thus prepared could be stored at 4°C. for several weeks without loss of thiamine activity.

All assays were carried out by the modification of the macro fermentation method of Schultz et al. ('42), as described by Scrimshaw and Stewart ('44). All values represent the average of at least two assay runs. When the agreement between these two runs was not satisfactory or when the values seemed to represent significant variations from the average, additional assays were carried out.

Four series of incubated eggs were studied utilizing eggs laid in May, 1943, January, February and April, 1944. Both sterile and fertile eggs were assayed in the first three series. Samples which were mixtures of four eggs were used throughout except for the study of the variation in individual unincubated eggs in series I. In series I and III controls were also kept at room temperature. Series III also had controls at 5°C. Series IV was planned primarily to check the previous findings on the thiamine content of fertile eggs during the last 5 days of incubation.

The variation in thiamine content of fresh hen eggs is shown in table 1. In series I the thiamine content of the whole egg varied from 62 to 93 μg./100 gm. When this was calculated on the basis of the total thiamine per egg, the variation was still from 35 to 56 μg. per egg. Presumably the thiamine in the eggs varied more in the 1944 assays because the four egg samples of series II and III show about the same variation as the one egg samples of the previous spring. The average thiamine content was observed to vary from one series to the next. The May, 1943, (series I) eggs were distinctly lower in the thiamine content than those of the April, 1944 (series IV), being 79 μg./100 gm. as compared with 110 (table 2).

The results of assays on developing eggs are recorded in table 2. Although considerable variation was found within a series, no significant decrease in the total thiamine content of the egg was apparent. In

series I, II, and III the 20- and 21-day values are lower than the 15- and 18-day values. However, they are still within the range of variation seen in the unincubated samples. In series IV the 16- and 20-day values were checked by repeated assays on duplicate samples. The average values of 118 and 106 μg./100 gm. of whole egg fail to indicate a significant decrease in the total thiamine content.

TABLE 1

Variation in thiamine content of fresh hen eggs.

SAMPLE	WT. EGG MINUS SHELL	SERIES I SINGLE EGG SAMPLES		SERIES II 4 EGG SAMPLES	SERIES III 4 EGG SAMPLES
		Thiamine/100 gm. egg	Thiamine per egg	Thiamine/100 gm. egg	Thiamine/100 gm. egg
	gm.	μg.	μg.	μg.	μg.
1	52.7	93	56.0	94	81
2	50.0	62	35.2	75	84
3	55.0	70	44.1	95	70
4	45.3	86	45.2	100	88
5	51.2	77	45.4	76	103
6	47.5	89	48.1	88	74
7	49.3	85	48.5	..	85
8	52.2	73	43.8	..	86
9	49.0	80	45.4	..	85
10	54.8	77	47.9	..	..
Average	50.7	79	45.9	88	84

TABLE 2

Thiamine content of fertile incubated eggs.

DAYS INCUBATED	THIAMINE CONTENT PER 100 GM. OF EGG (SAMPLES OF 4 EGGS)			
	Series I	Series II	Series III	Series IV
	μg.	μg.	μg.	μg.
fresh eggs	79	88	84	110
1	78	..	..	..
3	71	..	..	..
4	..	85	..	..
5	..	..	80	..
6	94	90	..	..
9	85	63	..	..
10	..	..	90	100
12	83	108	..	..
15	69	95	104	..
16	..	..	..	118
18	..	80	..	..
20	68	..	76	106
21	..	65	..	..

Similarly, no significant change could be detected in sterile eggs which were kept in the incubator with the developing ones (table 3). Control eggs kept at room temperature and at 5°C. likewise showed no decrease (table 3). The apparent increase in the thiamine content of the 20-day eggs in these cases is not significant.

TABLE 3

Thiamine content of non-developing eggs.

DAYS STORED	THIAMINE CONTENT PER 100 GM. OF EGG (SAMPLES OF 4 EGGS)			
	Sterile incubated		Room temperature	Cold room 5°C.
	Series I	Series III	Series III	Series III
	μg.	μg.	μg.	μg.
0	79	84	84	84
3	82	..	..	..
9	72	..	..	..
12	70	..	..	..
15	78	92	85	78
20	80	100	112	100

SUMMARY AND CONCLUSIONS

The total amount of thiamine in hen eggs assayed by the macro fermentation method does not change during incubation. This differs somewhat from the conclusion of Westenbrink and Van Leer ('41) that thiamine disappears during incubation. The discrepancy probably lies in the fact that these workers assayed only the yolk and the embryo. Perhaps the thiamine content of the extra embryonic structures and fluids is sufficient to make up the difference reported by these workers.

It was also found that the thiamine content does not change in sterile eggs kept at incubation temperatures for 3 weeks or in eggs kept for this period at room temperatures and at 5°C.

LITERATURE CITED

SCHULTZ, A. S., L. ATKIN AND C. N. FREY 1937 A fermentation test for vitamin B. II. J. Am. Chem. Soc., vol. 59, p. 2457.

———— 1942 Determination of vitamin B_1 by yeast fermentation method. Ind. Eng. Chem. Anal. Ed., vol 14, p. 35.

SCRIMSHAW, N. S., AND W. B. STEWART 1944 Use of the macro fermentation method for thiamine assay. J. Biol. Chem., vol. 155, p. 79.

SNELL, E. E., AND E. QUARLES 1941 The effect of incubation on the vitamin content of eggs. J. Nutrition, vol. 22, p. 483.

SUOMALAINEN, P. 1939 a Vitamin A and carotene in the hen egg during hatching. Suomen Kemistelehti, vol. 12B, p. 30. Chem. Abst., vol. 34, p. 145.

———— 1939 b Synthesis of ascorbic acid (vitamin C) in the hen egg during hatching. Ann. Acad. Sci. Fennicae, vol. A53, no. 8. Chem. Abst., vol. 34, p. 1724.

WESTENBRINK, H. G. K., AND I. S. VAN LEER 1941 The aneurine metabolism of the hen egg during development and incubation. Arch. Neerland. physiol., vol. 25, p. 544. Chem. Abst., vol. 37, p. 3485.

WILLIAMS, R. J., A. TAYLOR AND V. H. CHELDELIN 1941 Changes in the "B vitamin" content of tissues during development. Univ. Texas Publication no. 4137, p. 61.

WOOLLEY, D. W. 1942 Changes in the form of inositol during the incubation of eggs. Proc. Soc. Exp. Biol. Med., vol. 49, p. 540.

THE EFFECT OF THIAMINE DEPLETION AND RESTORATION OF MUSCULAR EFFICIENCY AND ENDURANCE [1]

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TWO FIGURES

(Received for publication May 25, 1944)

Since the early months of 1941 this laboratory has been carrying on experiments upon human subjects designed to bring out dietary factors which can influence to a measurable degree muscular efficiency or endurance or both. The Krogh bicycle ergometer and the Benedict Universal respiration apparatus provided the means of measuring the work done and the energy metabolism. One misses from most of the recent publications on this subject precise data on either of these items.

It was believed that the most favorable conditions for demonstrating alterations in efficiency and endurance would include the following: (1) The load and rate of work should be kept constant from day to day. (2) Likewise no dietary factor except the one under investigation should be allowed to vary significantly. (3) The subject should perform the identical work test time after time through, (a) a period of depletion of the vitamin by stages to very low levels until deterioration of performance became clearly evident. With intelligent and conscientious subjects one should not disregard subjective evidence of this state of deficiency and once it was established by unmistakable objective (and subjective) signs it should not be prolonged but should merge into (b) a period of gradual restoration until no further improvement could be discerned.

Any ideal series of experiments could readily be marred by the selection of uncooperative subjects or by an "edgy" relationship between operator and subject. The few men who figure most prominently in this particular study were selected from ten or twelve young men who were tried. Being of the more intelligent and more coopera-

[1] This study was supported by a grant from the Continental Baking Company of New York City and is taken from a thesis presented by J. W. A. in partial fulfillment of the requirements for the Doctorate of Philosophy.

tive type of students they were genuinely interested to learn their own capacities and to score a "perfect run" every day. They were also on the best of terms with the operator (J. W. A.) who encouraged them with praise when it was deserved and made them feel they were partners in the investigation.

The early papers on the role of thiamine in muscular work in man (McCormick, '40; Gounelle, '40; Morell, '40; Kraut and Droese, '41; Droese, '42 and others) apparently were optimistic to say the least. When a factor proves to be indispensable, a natural but uncritical attitude often is that above the level of mere sufficiency one should find more and more superior performance. Recent results have dispelled this concept concerning thiamine in relation to muscular work.

That thiamine is important in relation to exercise is seen clearly in the following investigations. Restriction of thiamine to 0.15 gm. daily for female patients in a state hospital, as reported by Williams, Mason, Wilder and Smith ('40, '42), produced after several months both physical and mental deterioration, the rate of appearance and severity of which definitely were augmented by physical exertion. Standardized tests on a chest exerciser were not borne so well as on a normal diet.

Further evidence of such a relationship is found in the work of Egaña et al. ('42) who investigated the effects on seven healthy physicians of a diet deficient in B-complex vitamins but adequate in calories and proteins for periods up to 4 weeks. They report moderate deterioration of physical fitness in exhaustive exercise and particularly poor recuperation between repeated periods of such work. The duration of an up-hill run which the subjects could tolerate was abnormally short in two of the three fittest subjects. At times the oxygen absorption was found to increase slightly as deficiency progressed and the authors inferred from this that the mechanical efficiency was impaired, but the authors did not center their attention on this phase of the work and apparently did not feel that impaired efficiency in the technical sense was demonstrated. Johnson et al. ('42) studied the effects of the same order of B-complex deficiency on ten men doing heavy labor. Marked deterioration in physical fitness made its appearance in 6 days. Brewer's yeast [2] in 18-gm. doses daily completely relieved all symptoms of deficiency and the physical deterioration within a week. The authors believe they definitely showed that thiamine alone of the B-complex factors will not maintain full physical efficiency.

[2] Fleischmann's no. 2019.

Of a quite different tenor are the experiments of Keys and Henschel ('41, '42) who have made extended observations on soldiers in which the amount of work seems to have been adequately measured but there is some doubt from their brief reports as published of the adequacy of measurement of respiratory metabolism. Their conclusion is that neither in brief, extreme exercise nor in prolonged, severe exercise and semi-starvation was there an indication of any effects, favorable or otherwise, of large daily supplements of thiamine chloride, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, and ascorbic acid on muscular ability, endurance, resistance to fatigue, or recovery from exertion. In another communication Keys et al. ('43) concluded that no benefit of any kind was observed to be produced by intakes of more than 0.23 mg. of thiamine per 1000 cal. of the diet (0.7 mg. for the average person daily). Even at the lowest level, muscular, neuro-muscular, cardio-vascular, psycho-motor and metabolic functions were in no way limited.

A third group of workers, Foltz, Ivy and Barborka ('42) have made observations both on the effects of administration of extra B-complex vitamins and on depleted states (Barborka et al. '43). The first study demonstrated that intravenous injection of B-complex vitamins did not influence recovery from fatigue or increase muscular efficiency in persons on adequate diets. The second report showed that diets deficient in B-complex vitamins decrease work output in trained subjects; that early mild states of malnutrition with no objective evidence of deficiency disease do prevent maximum work efficiency; and that subjective symptoms of fatigue, irritability, lack of pep, anorexia and leg pains all disappeared and work output returned to normal or better within a few days following supplementation of the diet with B-complex vitamins (yeast concentrate).

EXPERIMENTAL

Experiments on the ergometer and respiration apparatus were conducted both in the post-absorptive and the post-cibal states. Briefly, each day's experiment consisted: (1) in determining the resting energy metabolism of the subject for a 10-minute period during which he sat without pedalling on the ergometer; and (2) in determining the metabolism for a 15-minute period of work and recovery, the subject producing work at the rate of 455-kg.-meters per minute for 6 minutes of this period. This apportionment of time between active work and recovery from the oxygen debt was established after considerable study of the recovery of alveolar CO_2 as well as pulse and respiration rates. Respiratory quotients were obtained and the ratio of work (in calories)

to energy expenditure due to work was calculated as net efficiency. If an endurance experiment was to be done on the same day it usually followed immediately after the muscular efficiency test. For the endurance test the subjects worked at rates varying between 1180– and 1475-kg. meters per minute, until they became exhausted, but did not breathe into the respiration appparatus. That the subject really was exhausted was readily established once the subject's characteristic performance was known.

Repeated checks were made of the Benedict Universal apparatus by combustion of alcohol in the system. Twenty such experiments gave an average R. Q. for ethyl alcohol of 0.666 ± .04.

The low vitamin diet used was selected especially for its low content of thiamine but, in all probability it was low in some of the other B-complex vitamins as well. A sample day's diet is given in table 1. The total number of calories was about 3000, and the percentages of protein, fat, and carbohydrate were 12, 41, and 47, respectively. Approximately 75 mg. of ascorbic acid were added to the diet daily. The estimated quantity of thiamine was 0.27 mg.[3] daily. A still lower thiamine diet (no. 3) was obtained later when the unenriched white bread was replaced with soda crackers. Analysis placed the level at 3 to 7 μg. per 100 gm. of the food, which meant that the total day's supply of thiamine in the modified diet was less than 0.15 mg. Also no butter fat was included in the modified diet and this resulted in an increased carbohydrate percentage.

One hundred and eighty gm. of a peeled-wheat bread,[4] from flour produced by the flotation process of Earle (Murlin, Marshall and Kochakian, '41), were substituted first for the unenriched white bread in diet no. 2 when it was desired to add B-complex vitamins to the diet in a natural product. This bread contains a greater quantity of the vitamins than the usual "whole wheat" bread made from flour containing less of the whole grain (Sealock and Livermore, '43). Subsequently the peeled-wheat bread was doubled in quantity, and, during this period, the carbohydrate calories from other foods in the diet were decreased to compensate for the carbohydrate calories added by the extra 180 gm. of the peeled-wheat bread. The quantity of thiamine added by the substitution of the 180 gm. of the peeled-wheat bread was about 0.55 mg. daily, or 1.0 mg. from 360 gm. of the bread.

[3] All determinations of thiamine were made by Dr. N. S. Scrimshaw using the fermentation method of Schultz, Atkin and Frey ('42) as modified by Scrimshaw and Stewart ('44).

[4] This bread, known to the trade as "Staff," was supplied by the Continental Baking Company of Rochester, N. Y.

TABLE 1

Low thiamine diet no. 2.

FOOD	Weight	Protein	CALORIES FROM Fat	Carbohydrate	Thiamine
	(gm.)				(μg.)
Breakfast					
V.C.B. preparation [1]	10	..	..	25.0	..
White bread	60	22.3	3.8	102.7	27
Butter fat	25	..	198.0	..	..
Honey	33	0.4	..	55.0	6
Cream (40%)	100	9.8	165.6	11.5	..
Sugar	20	..	..	80.0	..
Egg white	90	49.2	..	..	..
Peaches	100	2.9	..	45.1	60
Coffee (1 cup)	..	..	..	..	..
Total		84.6	367.4	319.3	93
Lunch					
White bread	60	22.3	3.8	102.7	27
Butter fat	25	..	198.0	..	..
Salmon	100	88.6	108.0	..	..
Polished rice	55.6	18.2	1.4	180.4	18
Beets	100	7.0	..	41.0	18
Olives	40	16.4	81.0	15.6	..
Honey	33	0.4	..	55.0	6
Pears	100	3.6	8.0	217.2	58
Total		156.5	400.2	611.9	127
Dinner					
V.C.B. preparation	10	..	..	25.0	..
White bread	60	22.3	3.8	102.7	27
Butter fat	25	..	198.0	..	..
Sugar	30	..	..	120.0	..
Cream cheese	46	48.4	146.0	4.4	19
Cranberry jelly	50	..	..	143.5	..
Honey	33	0.4	..	55.0	6
Olives	40	16.4	81.0	15.6	..
Gelatin	5	20.5	..	..	..
Total		108.0	428.8	466.2	52
Grand total		349.1	1196.4	1397.4	272

[1] The V.C.B. preparation is a product manufactured by the Hilker and Bletsch Co., Chicago. It is stated as containing cane sugar, dextrose, concentrated fruit juices, essential oils, citric acid, artificial food color, and ascorbic acid, the latter in the amount of 3000 mg. for every 28 ounces, or about 3.8 mg. for every gram of the preparation.

TABLE 2

Work metabolism data of L. H. during thiamine and B complex study.[1]

DATE 1942	RESPIRATORY QUOTIENTS			CC. CO_2/CC. O_2/MIN.		NET EFFICIENCY	ENDURANCE	
	Rest	Work and recovery	Excess	Rest	Work			
						%	*kg. m.*	*min. sec.*
Normal diet								
4/10	.81	.86	.89	206/254	1235/1415	18.39	4001	3′ 23″
4/13	.87	.88	.89	198/229	1227/1387	18.01	4268	3′ 36″
Average	.84	.87	.89	202/242	1231/1401	18.20		
Low thiamine diet								
4/14	.87	.95	1.00	216/249	1278/1310	19.74	4052	3′ 26″
4/15	.96	.88	.83	231/242	1153/1347	19.57	4248	3′ 36″
4/16	.79	.94	1.02	220/278	1197/1207	22.08	4858	4′ 7″
4/17	.76	.91	1.01	211/277	1176/1233	21.62	4721	4′ 0″
4/18	.92	.94	.95	218/238	1214/1283	20.11	5074	4′ 18″
4/19	.83	.98	1.10	233/280	1246/1203	22.00	4937	4′ 11″
4/20	.84	.96	1.03	222/263	1106/1121	23.90	4799	4′ 4″
4/21	.73	.88	.97	189/259	1191/1290	20.10	4838	4′ 6″
4/22	.81	.90	.96	206/254	1181/1271	20.64	4799	4′ 4″
4/23	.80	.88	.94	197/247	1171/1287	20.32	4759	4′ 2″
4/24	.83	.90	.95	206/248	1154/1250	21.02	5625	4′ 46″
4/25	.88	.94	.98	199/226	1191/1242	20.58	5506	4′ 40″
4/26	.83	.91	.95	215/258	1150/1238	21.38	5290	4′ 29″
4/27	.88	.94	.97	212/241	1203/1265	20.51	5330	4′ 31″
4/28	.85	.91	.95	223/260	1149/1232	21.57	5564	4′ 43″
4/29	.82	.96	1.06	210/255	1177/1230	22.05	5605	4′ 45″
4/30	.73	.91	1.03	190/262	1191/1238	20.83	5762	4′ 53″
5/1	.69	.89	1.01	181/261	1211/1277	20.20		
5/2	.77	.84	.90	210/274	1078/1244	21.65	5999	5′ 5″
5/3	.78	.88	.94	207/265	1147/1261	20.38	5447	4′ 37″
5/4	.75	.89	.98	186/246	1199/1281	20.45	5564	4′ 43″
5/5	.87	.90	.92	197/225	1195/1312	19.09	5447	4′ 37″
5/6	.83	.92	.98	192/231	1190/1254	20.30	5389	4′ 34″
5/7	.72	.90	1.01	174/242	1202/1258	20.73	5153	4′ 22″
5/8	.85	.91	.94	213/249	1161/1260	20.84	5132	4′ 21″
5/9	.87	.92	.92	214/245	1133/1211	21.46	4739	4′ 1″
5/10	.82	.95	1.03	209/254	1267/1286	19.99	4642	3′ 56″
5/11	.68	.92	1.04	155/228	1296/1323	19.49	4190	3′ 33″
5/13	.73	.94	1.09	200/273	1265/1254	20.73	4246	3′ 36″
5/15	.87	.95	1.02	215/247	1347/1289	21.28	3942	3′ 22″
Average	.81	.92	.98	205/253	1194/1259	20.82		
Replacement of unenriched white bread with peeled wheat bread (180 gm.).								
5/16	.87	.87	.88	205/235	1141/1304	20.29	4097	3′ 26″
5/17	.80	.82	.83	199/248	1063/1289	20.73	4284	3′ 37″
5/19	.84	.93	.99	213/255	1150/1218	21.50	4223	3′ 32″
5/20	.81	.90	.95	201/249	1164/1269	20.44	4187	3′ 32″
5/21	.78	.95	1.06	199/256	1228/1227	21.01	4781	4′ 4″
5/22	.82	.93	1.00	201/244	1233/1277	20.32	5346	4′ 31″
5/23	.79	.98	1.11	193/246	1281/1230	20.66	5281	4′ 25″
5/24	.72	.89	1.03	207/289	1168/1223	22.05	4804	4′ 4″
Average	.80	.91	.98	202/253	1179/1255	20.88		
Peeled wheat bread doubled (360 gm.)								
5/25	.83	.93	1.00	214/259	1218/1261	20.81	4907	4′ 8″
5/26	.84	.92	.96	218/258	1153/1218	21.50	5540	4′ 43″
5/27	.88	.94	.97	208/236	1146/1201	21.42	6246	5′ 13″
5/28	.94	.95	.95	217/230	1224/1289	20.16	6521	5′ 36″
5/31	.73	.87	.95	177/243	1175/1292	19.73	7088	5′ 55″
6/1	1.03	.95	.91	237/230	1132/1218	20.90	8566	7′ 9″
Average	.88	.93	.96	212/243	1175/1247	20.75		

[1] All runs were made by this subject in the post-absorptive state.

Experiments were conducted with subject L. H., while on the low-thiamine diet no. 2, for a period of 28 consecutive days and 2 days at 1-day intervals (table 2 and fig. 1). At the end of this period the 180 gm. of peeled-wheat bread were substituted for the unenriched white bread in the diet and experiments were continued for 9 days. When the quantity of peeled-wheat bread was doubled, the experiments were continued for 8 days more. Corresponding periods for subject C. B.

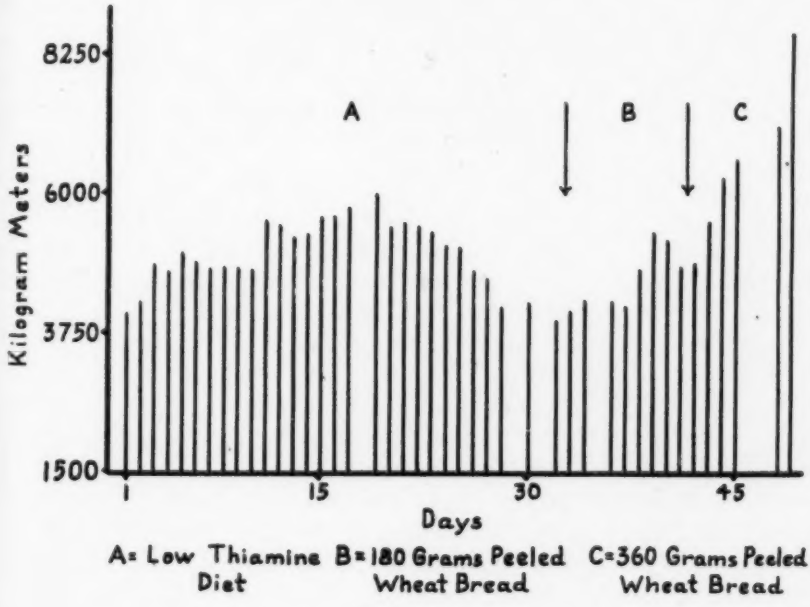

Figure 1

lasted 26, 8, and 7 days, respectively. Subsequent modifications in the study with C. B. consisted in the addition to the diet of thiamine hydrochloride, pyridoxine hydrochloride, and riboflavin for short periods (fig. 2). During the periods when the synthetic vitamins were added there were already 360 gm. of peeled-wheat bread daily in the diet. Experiments with L. H. were conducted in the post-absorptive state only, while those with C. B. were conducted in both the post-absorptive and the post-cibal states.

Subsequent studies were made when, for a period of 12 days, L. H. and C. B. went on the low thiamine diet no. 3 modified by the replacement of the unenriched white bread with soda crackers. Peeled-wheat

bread in the amount of 360 gm. was substituted for the soda crackers, and this resulted in a further increased carbohydrate content of the diet, as well as the addition of the B-complex vitamins. L. H. remained on this regime for 10 days and C. B. for 6 days. Later modifications in the diet of L. H. consisted in the addition of thiamine hydrochloride and a high vitamin yeast concentrate [5] for short periods. Finally 360 gm. of a high-vitamin yeast [6] bread, made from 20% soybean flour were substituted for the soda crackers for a short period. Efficiency measurements were conducted with both subjects in the post-cibal state. A third subject, J. S., was placed for 8 days on the low thiamine diet no. 3 used in these later experiments. Subsequently thiamine hydrochloride, riboflavin, and pyridoxine hydrochloride separately were added to his diet for short periods, and measurements, including endurance experiments, were made.

RESULTS

The muscular efficiency and endurance data, obtained in the first study with L. H., are given in full in table 2. It is evident from the table that efficiency and endurance increased — the former rather steeply but not smoothly for 7 days and the latter steadily during a period of 19 days (training effect) in spite of the deficiency; but from this point to the end of the low thiamine diet period, there was an equally steady decrease in the latter criterion of performance with no change in the former. The average net efficiency was 20.82%. The average net efficiency for the period when the 180 gm. of the unenriched white bread were replaced by the 180 gm. of the peeled-wheat bread was 20.88%, while endurance improved more than 25% in work and 1 minute in time. The average efficiency for the period when the quantity of peeled-wheat bread was doubled was 20.75%, but the endurance more than doubled over the deficiency period both in work and time. Thus the net efficiency, as measured during these post-absorptive experiments, remained practically the same, except for the first few days, during the entire 49 days, while endurance greatly improved after restoration of some factor or factors supplied by the peeled-wheat bread.

The same results were obtained in the post-absorptive experiments with C. B. as in those with L. H.: there was no increase in efficiency as a result of adding the B-complex vitamins in the form of the peeled-wheat bread. The average net efficiency for the low thiamine diet

[5] Fleischmann's.

[6] See Murlin, Marshall and Kochakian, '41, p. 579, for a description of this yeast.

was 19.79%, the average for the period with 180 gm. of the peeled-wheat bread substituted for the unenriched white bread was 19.12%, and the average with 360 gm. peeled-wheat was 19.20%. The inclusion in the diet of thiamine hydrochloride, pyridoxine hydrochloride, or riboflavin did not increase the efficiency in the post-absorptive state. Endurance, however, improved for the first 10 days (training effect) then declined, to a point much below the starting level, to the end of deficiency (fig. 2). Peeled-wheat bread raised it only for the first 3 days when fed at the lower level, and not so much as with L. H. at the higher

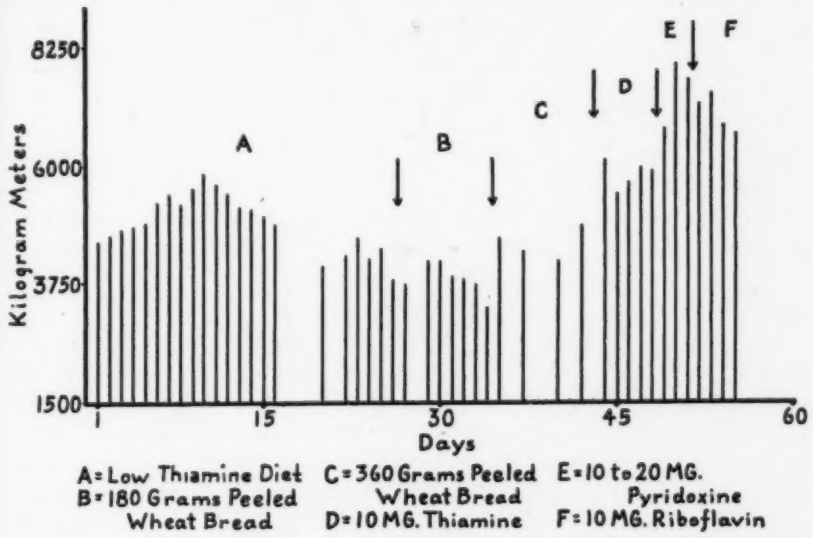

Figure 2

level. Addition of 10 mg. thiamine, however, raised endurance to a point equal relatively to that reached by L. H. on 360 gm. of the bread — a result indicating clearly that C. B. did not obtain the full benefit of the bread, or that his requirement for thiamine was much higher than that of L. H.

The average net efficiency of C. B. for the post-cibal, low thiamine experiments was 20.10% which was only a slight increase over that of the post-absorptive experiments. On 180 gm. of the peeled-wheat bread it was 21.27%, a relative increase of 5.5%; and when the peeled-wheat bread was doubled it was 20.95%, an increase of 4.2%. The addition of the synthetic vitamins resulted in no significant increases over the period when 180 gm. of the bread were ingested.

The post-cibal net efficiencies of C. B. are arranged in table 3 as "low" thiamine" values and "peeled-wheat-bread" values. The latter include all those post-cibal experiments in which either 180 or 360 gm. of the peeled-wheat bread were incorporated in the diet. The difference between the averages is statistically significant, and it appears that whole wheat bread contributes some factor affecting efficiency. That the increase was not due to the post-cibal state per se is indicated by the fact that the average net efficiency of the post-cibal experiments on the low thiamine diet was only slightly increased above the average efficiency of the post-absorptive experiments on the same diet. That the increase in efficiency was not due to the extra carbohydrate may be concluded, because carbohydrate foods were reduced in the diet to compensate for the extra starch contributed by the 360 gm. of peeled-wheat bread.

As the increase in efficiency with C. B. was of small magnitude, additional confirmatory experiments were conducted 6 months later when C. B. and L. H. were placed on the diet lowest in thiamine containing not more than 0.15 mg. daily. Experiments were conducted in the post-cibal (p. c.) as well as the post-absorptive state. Nearly all of the former were begun 1 hour after lunch. The average p. c. efficiency of L. H. on this diet was 21.05% (7 days). When 360 gm. of peeled-wheat bread (6 days), a yeast concentrate (1 day), and a high-vitamin yeast bread made with 20% soybean flour (2 days) were added separately to the diet, the average p. c. efficiency was 21.44%. The difference is not statistically significant. The average percentage p. c. net efficiency during the period of thiamine administration was 20.92%, practically the same as after lowest thiamine. The average p. c. efficiency of C. B., when the peeled-wheat bread was added to the diet, was 21.30%, in contrast with 19.68% on the low thiamine diet. This difference has statistical significance (p value 0.01) as in the former experiments with the same subject (table 3), when the p value was 0.02.

The average respiratory quotients of L. H. and C. B. during the thiamine and B-complex study are given in table 4. That carbohydrate oxidation was not decreased during the 32 days of the deficiency is evident from the excess respiratory quotients. A marked difference was apparent in the character of the metabolism during muscular work of the two subjects. The excess quotients of L. H. indicated almost pure carbohydrate combustion, while those of C. B. (average = 0.89) indicated that fat or protein contributed to the energy of work.

Some of the subjective symptoms noted by C. B. and L. H., while on the low thiamine diet, were fatigue, anorexia, slight diarrhea, in-

ability to concentrate well, and abdominal "fullness." These symptoms were observed first in about 10 days to 2 weeks of the deficiency. They progressed in intensity and at the end of the deficiency period nausea occurred in both subjects during the endurance rides. When 180 gm. of the peeled-wheat bread were added to the diet L. H. felt better but

TABLE 3

Post-cibal percentage net efficiencies of C.B.

	"LOW THIAMINE"	"PEELED-WHEAT BREAD"
	19.40	22.19
	21.87	21.11
	19.81	20.61
	22.28	21.16
	20.36	20.04
	19.91	21.85
	20.13	20.15
	19.75	22.46
	19.76	20.13
	20.56	20.99
	19.54	20.04
	18.67	21.41
	19.29	
Average	20.10 ± 1.00	21.01 ± 0.85

Note — Fisher's p value of difference = .02.

TABLE 4

Average respiratory quotients of L.H. and C.B.

PERIOD	REST R. Q.		WORK AND RECOVERY R. Q.		EXCESS R. Q.	
	L.H.	C.B.	L.H.	C.B.	L.H.	C.B.
1. Low thiamine diet no. 2 (0.27 mg. thiamine daily)	.81	.82	.92	.88	.98	.89
2. Substitution of 180 gm. of peeled-wheat bread (0.55 mg. thiamine extra)	.80	.79	.91	.85	.98	.89
3. 360 gm. of peeled-wheat bread (1.10 mg. thiamine extra)	.88	.87	.93	.86	.96	.87

C. B. did not. When 360 gm. of the bread were added both men felt decidedly better, and their mental attitudes improved.

The third subject J. S., rather corpulent in build but very willing and cooperative in temperament, participated in this study but his efficiency and endurance proved to be so low that only a short series

of experiments were run with him. Neither 10 mg. thiamine chloride nor 10 mg. riboflavin nor 25 mg. pyridoxine added as pure substances to the low thiamine diet raised his performance in any respect, probably because the earlier depletion period was not long enough.

SUMMARY AND CONCLUSIONS

1. Muscular endurance was greatly decreased on a diet which was low in the B-complex vitamins (thiamine 0.27 mg. daily). The effect was observed first in about 10 days to 2 weeks of the deficiency. When B-complex vitamins were added to the diet in the form of a natural source, such as a 98% whole wheat bread in amounts containing 0.55 and 1.10 mg. thiamine, the muscular endurance was increased significantly.

2. The inclusion of thiamine hydrochloride in pure form in a low B-complex diet resulted in marked improvement in endurance ability. Pyridoxine (in one experiment) had a similar effect, but riboflavin had none.

3. The inclusion of B-complex vitamins in a diet already adequate in these vitamins did not result in increased muscular endurance.

4. The muscular efficiency in the post-absorptive state remained the same for moderate work, even though the body was low in the B-complex vitamins, particularly thiamine, for a period of at least a month. The addition of B-complex vitamins, including thiamine, in such amounts that the daily requirements were met, did not increase the muscular efficiency in the post-absorptive state.

5. A small significant increase in muscular efficiency for moderate work was obtained with one subject in two different studies, when the B-complex vitamins were supplied to the diet by the peeled-wheat bread, and the experiments were conducted in the post-cibal state. A small increase, which was not significant, was obtained with another subject when a shorter series of experiments were conducted in the post-cibal state. It is concluded that any increase in efficiency which occurs with administration of the B-complex vitamins, on low B-complex diets of at least a month's duration, is in the post-cibal and not the post-absorptive state. This suggests that the entrance into the circulation and access to the muscles simultaneously of fuel and vitamins which promote release of energy are important. The administration of thiamine alone did not result in any increase in efficiency in the post-cibal state.

6. Carbohydrate oxidation, as a source of energy for moderate muscular work, was not decreased during a period of low B-complex intake for a period at least as long as a month.

7. The subjective symptoms of individuals on the diet, selected especially for its low thiamine content, and their improvement when B-complex vitamins were added to the diet in the peeled-wheat bread, have been noted as similar to those described by Williams, Wilder and associates ('40, '42). We confirm Johnson and associates ('42) in finding that the B-complex vitamins, given in these experiments as peeled-wheat bread, may improve efficiency and endurance whereas thiamine improves endurance only.

LITERATURE CITED

BARBORKA, C. J., E. E. FOLTZ AND A. C. IVY 1942 The use of double work periods in the study of fatigue and the influence of caffeine on recovery. Am. J. Physiol., vol. 136, p. 79.

———— 1943 Relationship between vitamin B complex intake and work output in trained subjects J. Am. Med. Assn., vol. 122, p. 717.

DROESE, W. 1942 Die Wirkung von Traubenzucker und Traubenzucker B_1-Kombinationen auf die Leistungsfähigkeit bei Hitzarbeit. Arbeitsphysiologie, vol. 12, p. 124.

EGAÑA, E., R. E. JOHNSON, R. BLOOMFIELD, L. BROUHA, A. P. MEIKELJOHN, J. WHITTENBERGER, R. C. DARLING, C. HEATH, A. GRAYBIEL AND F. CONSOLAZIO 1942 The effects of a diet deficient in the vitamin B complex on sedentary men. Am. J. Physiol., vol. 137, p. 731.

FOLTZ, E. E., A. C. IVY AND C. J. BARBORKA 1942 Influence of components of the vitamin B complex on recovery from fatigue. J. Lab. and Clin. Med., vol. 27, p. 1396.

GOUNELLE, H. 1940 Action de la vitamin B_1 dans l'exercise musculaire et la prevention de la fatigue. Bull. et Mem. Soc. Med. de Hsp. de Paris, vol. 56, p. 255.

JOHNSON, R. E., R. C. DARLING, W. H. FORBES. L. BROUHA, E. EGAÑA AND A. GRAYBIEL 1942 The effects of a diet deficient in part of the vitamin B complex upon men doing manual labor. J. Nutrition, vol. 24, p. 585.

KEYS, A., AND A. F. HENSCHEL 1941 High vitamin supplementation (B_1, nicotinic acid and C) and the response to intensive exercise in U. S. Army infantrymen. Am. J. Physiol., vol. 133, p. 350.

———— 1942 Vitamin supplementation of U. S. Army rations in relation to fatigue and the ability to do muscular work. J. Nutrition, vol. 23, p. 259.

KEYS, A., A. F. HENSCHEL, O. MICKELSEN AND J. M. BROZEK 1943 The performance of normal young men on controlled thiamine intakes. J. Nutrition, vol. 26, p. 399.

KRAUT, H., AND W. DROESE 1941 Ernährung und Leistungsfähigkeit. Angew. Chem., vol. 54, p. 1.

MCCORMICK, W. J. 1940 Some clinical indices of vitamin B deficiency. Med. Record, vol. 152, p. 439.

MORELL, T. 1940 Ermüdungsbekämpfung durch körpereigene Wirkstoffe. Deutsch. Med. Wochenschr., vol. 66, p. 398.

MURLIN, J. R., M. E. MARSHALL AND C. D. KOCHAKIAN 1941 Digestibility and biological value of whole wheat breads as compared with white bread. J. Nutrition, vol. 22, p. 573.

SEALOCK, R. R., AND A. H. LIVERMORE 1943 The vitamin B complex of peeled-wheat bread. J. Nutrition, vol. 25, p. 265.

SCHULTZ, A. S., L. ATKIN AND C. N. FREY 1942 Determination of vitamin B_1 by yeast fermentation method. Ind. and Eng. Chem., Anal. Ed., vol. 14, p. 35.

SCRIMSHAW, N. S., AND W. B. STEWART 1944 Use of the macro fermentation method for thiamine assay. J. Biol. Chem., vol. 155, p. 79.

WILLIAMS, R. D., H. L. MASON AND R. M. WILDER 1942 The minimum daily requirement of thiamine of man. J. Nutrition, vol. 25, p. 71.

WILLIAMS, R. D., H. L. MASON, R. M. WILDER AND B. F. SMITH 1940 Observations on induced thiamine (vitamin B_1) deficiency in man. Arch. Int. Med., vol. 66, p. 785.

THE EFFECT OF INSTITUTIONAL COOKING METHODS ON THE VITAMIN CONTENT OF FOODS

I. THE THIAMINE CONTENT OF POTATOES [1]

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(Received for publication May 27, 1944)

Since a significant number of persons are eating daily one or more meals which have been prepared in large quantity, it is desirable to know the extent to which the nutritive value of food may be affected by the processes involved in institutional cooking. Accordingly, a study was undertaken to determine the losses of certain nutrients in foods prepared in large quantity.

The foods were obtained at the College dining hall which, at the beginning of the study, served three meals a day to approximately 500 college students. Six months later the students were replaced by 750 Army Air Corps students, the food services being taken over by the Army. The change in management did not necessitate a change in either personnel, equipment, techniques of cookery, or types of food served; therefore it was possible for the study to continue without interruption.

Inasmuch as the white potato forms a substantial part of the diet of many persons in the United States, it was one of the first vegetables studied. This paper reports data obtained on the thiamine content of the potato at different steps in preparation for serving.

EXPERIMENTAL

The potatoes studied were of the Green Mountain variety, grown in the Connecticut River Valley. The analyses were begun in October, 1942, and carried on throughout the winter months, ending in May, 1943.

Preparation of the potatoes. The potatoes for both the noon and evening meal were peeled between 9 and 10 o'clock in the forenoon, approximately 500 lbs. being prepared each day. An electric abrasive peeler was used. The potatoes which were served for the noon meal

[1] Contribution no. 515 of the Massachusetts Agricultural Experiment Station.

were washed and cooked immediately, whereas those used for the evening meal were placed in a vat and soaked for approximately 6 hours in tap water, which was slowly running into and out of the vat. Upon removal from the water the potatoes were placed in perforated metal trays in a steam-oven and subjected to steam at a temperature of approximately 225°F. for 1 hour. During this cooking process whatever steam was condensed was automatically removed from the bottom of the steam-cabinet and discarded.

If the potatoes were to be mashed they were mashed immediately by whipping in an electric mixer. Fat, salt, pepper, and milk in the ratio of 5 lbs. to 50 lbs. of potatoes were added during the whipping. The entire mashing process took about 5 minutes.

During the interval between the completion of the cooking and the serving, the potatoes, either whole or mashed, were placed in trays in a steam warming-oven at a temperature of 157°F. The time of holding ranged from 50 to 120 minutes.

Sampling. Samples of the potatoes for analysis were taken at the following steps in preparation: (1) pared, (2) soaked 6 hours in tap water, (3) steamed, (4) mashed, (5) steamed and held for 1½ hours at 157°F. and (6) mashed and held for 1½ hours at 157°F. For the analysis of the whole potato, either in the raw or steamed condition, wedge-shaped slices about ¼ inch thick at the periphery were cut from the bud-end to the stem-end of the potato in order to obtain a representative sample from each tuber. For each sample slices were taken from 20 to 25 potatoes. These slices were cut in small pieces and aliquots taken for analysis. The mashed potatoes were sampled immediately upon the cessation of movement of the beater, small samples being removed from various areas until 600 to 700 gm. had been obtained.

All the sampling was done at the mess hall and the samples brought immediately to the laboratory where the analyses were carried out, the distance between the two buildings being about 300 feet. A period of approximately 15 minutes elapsed before the samples for thiamine determinations were placed in the extracting fluid. The weighings for the moisture determinations were made at the same time.

Thiamine determination. In the determination for thiamine the Hennessy and Cerecedo ('39) method as modified by Moyer and Tressler ('42) was used. For each sample 45 gm. of potatoes were taken and finely divided in a Waring Blendor with approximately 100 ml. of 0.1N H_2SO_4. The mixture was transferred to a 500 ml. flask and diluted with enough 0.1N H_2SO_4 to bring the volume to 350–400 ml.;

it was then heated on a steam bath for ½ hour and later cooled to below 50° C. Twenty-five millimeters of 2.5M sodium acetate, containing 0.6 gm. of clarase and 0.4 gm. of papain were then added, and the sample was incubated at 47°C. for 15 hours. After incubation, the samples were cooled, made to volume, and filtered. The base exchange and oxidation procedures were carried out in the usual manner.

Moisture. Moisture determinations were made on all samples in an electric oven set at 80°C. Computation of the losses in thiamine values were based on the dry weight of the potatoes.

RESULTS

Effect of soaking. The effect of soaking potatoes in running, cold water was studied in 9 lots of potatoes (see table 1). For the raw pared potatoes the thiamine content averaged 84 µg. per 100 gm.; whereas, following the soaking period it averaged 79 µg., indicating an average loss of 5%. That this loss was not due to chance alone, is seen in the statistical evaluation of the data (Student, '25), odds of 137 to 1 being obtained.

TABLE 1

Effect of prolonged immersion in water on thiamine content of potatoes.

LOT NO.	RAW, PARED			RAW, PARED, SOAKED			CHANGE IN THIAMINE CONTENT
	Moisture	Thiamine content		Moisture	Thiamine content		
		As exam.	Dry wt.		As exam.	Dry wt.	
	%	µg./100 gm.	µg./100 gm.	%	µg./100 gm.	µg./100 gm.	%
1	81.7	85	464	81.5	78	422	— 9.0
2	82.0	82	455	81.3	77	412	— 9.4
3	81.4	80	430	80.8	80	417	— 3.0
4	82.1	87	486	82.0	86	478	— 1.6
5	84.2	69	437	82.7	69	399	— 8.7
6	82.4	90	511	84.3	78	494	— 3.3
12	80.0	81	405	81.0	77	405	0.0
13	81.0	83	441	80.7	86	443	+ 0.4
14	83.5	96	583	84.0	83	518	— 11.1
Ave.	82.0	84	468	82.0	79	443	— 5.1

Effect of steam cooking. The effect of steam cooking was studied in 6 lots of potatoes (see table 2). The thiamine content averaged 99 µg. per 100 gm., this value being somewhat higher than the value of 84 µg. found for the 9 lots of potatoes on which the effect of soaking was studied. Following cooking, the thiamine content had decreased in

each of the six instances to a mean value of 89 μg. per 100 gm., the losses averaging 14.1%.

Effect of mashing. The effect of subsequent mashing on the thiamine content of the steam-cooked potato was studied in only 3 lots of potatoes; i. e., lots 8, 9, and 10. However, 5 other lots of potatoes (nos. 1 through 7) were analyzed for thiamine in both the raw and the mashed condition. The differences between the values for the 8 lots of the raw and the mashed potatoes represented losses which averaged 16.7%. This loss is slightly greater than the loss due to steaming alone. However, practically all of this difference may be accounted for by the dilution in thiamine which resulted from the addition to the potatoes of milk of lower thiamine content (35 μg. per 100 ml.)

Effect of holding in a steam-oven. The influence of holding the potatoes, either whole or mashed, at a temperature of 157°F. for 1½ hours is also shown in table 2. The losses in the thiamine content of the whole potato averaged 8.3% whereas those for the mashed potato averaged 6.4%.

DISCUSSION

The mean value for the losses in thiamine in whole potatoes cooked and served under institutional conditions exclusive of losses during soaking was 20.5%, whereas that for the mashed potatoes was 21.7%, a difference which is not significant. The major loss accompanied the cooking of the potato. Additional losses were incurred during the preliminary soaking of the potato and during holding of the potato in the steam-oven until serving time 1½ hours later, the latter loss being approximately the same whether the potatoes were whole or mashed. It is probable that the mashing process was without effect on the vitamin.

The literature is not replete with papers dealing with the destruction of thiamine in potatoes during cooking processes. Only two studies have been found dealing with foods prepared on an institutional basis. Nagel and Harris ('43) determined the losses of thiamine in foods cooked in quantities sufficient to serve 600 persons at each meal. They reported a 5% loss in the thiamine content of the potato during cooking alone and a 29% loss due to the entire cooking process and subsequent holding on a steam-table for approximately 3 hours. Heller et al. ('43) on analyzing the thiamine in potatoes served in one of the cafeterias at the Brooklyn Navy Yard found a loss of only 2 to 7%. In contrast to these studies are four studies on potatoes prepared for serving under laboratory or home conditions. Thus Wiegand ('38) found losses

TABLE 2

The loss in the thiamine content of potatoes due to steam-cooking, mashing and holding in a steam-oven.

LOT NO.	MOISTURE	THIAMINE CONTENT		MOISTURE	THIAMINE CONTENT			MOISTURE	THIAMINE CONTENT			TOTAL LOSS IN THIAMINE
		As examined	Dry weight		As examined	Dry weight	Loss		As examined	Dry weight	Loss in steam-oven	
	%	*µg./100 gm.*		%	*µg./100 gm.*		%	%	*µg./100 gm.*		%	%
		Raw, pared			Steamed 1 hr. at 225°F.				Held in steam-oven 1½ hrs. at 157°F.			
2 [1]	81.3	77	412	81.5	74	400	2.9	79.5	80	390	2.5	5.3
5 [1]	82.7	69	399	82.0	62	346	13.3	80.4	62	317	8.1	20.6
8 [2]	79.9	115	573	77.2	98	431	24.8	75.5	92	380	12.0	33.6
9 [2]	80.7	103	533	79.7	100	493	7.5	78.8	90	419	14.8	21.4
10 [2]	79.9	114	568	78.9	107	506	10.9	78.6	108	504	0.4	11.2
11	79.9	114	568	78.8	90	426	25.0	78.5	84	391	12.1	31.1
Ave.	80.7	99	509	79.7	89	434	14.1	78.5	86	400	8.3	20.5
		Raw, pared			Steamed 1 hr. at 225°F. Mashed immediately				Steam-oven 1½ hrs. at 157°F.			
1	81.5	78	422	82.0	74	411	2.6	79.1	81	390	5.1	7.6
3	80.8	80	417	80.6	71	366	12.2	80.0	66	347	4.6	16.8
4	82.0	86	478	81.5	83	450	5.8	80.9	82	430	4.4	10.0
6	84.3	78	494	82.2	65	364	26.3	81.7	67	362	0.6	26.7
7	82.4	78	443	80.9	69	361	18.5	79.5	67	327	9.4	26.2
8 [2]	79.9	115	573	78.8	100	470	18.0	76.9	92	400	15.5	30.2
9 [2]	80.7	103	533	77.5	94	419	21.4	79.3	81	391	6.6	24.6
10 [2]	79.9	114	568	78.2	88	405	28.7	78.2	84	385	4.9	32.0
Ave.	81.4	91	491	80.2	81	406	16.7	79.6	78	379	6.4	21.7

[1] These potatoes were soaked 6 hours before cooking.

[2] These 3 lots of mashed potatoes were analyzed for thiamine at the raw, steam-cooked, and the mashed stages.

of thiamine due to cooking ranging from 15 to 75% and averaging 34%. Aughey and Daniel ('40) reported a 16% loss in baked potatoes and a 20% loss in boiled potatoes from which the cooking water had not been discarded. In boiled potatoes from which the cooking water had been discarded, Lane et al. ('42) observed a loss in thiamine approximating 36%. In the tentative report of the Food and Nutrition Board ('43) the values for losses in thiamine are given as ranging from 10 to 36% in boiled potatoes, 16 to 35% in baked potatoes and 10 to 55% for all methods combined.

The raw potatoes used in this study contained thiamine within the range of values reported by other investigators. Thus, Lane et al. ('42) found 84 μg. per 100 gm. whereas Heller et al. ('43) reported values for five samples ranging from 53 to 90 μg. Wiegand's data ('38) for five varieties grown in Holland indicate a range of values from 48 to 110 μg. per 100 gm. Aughey and Daniel ('40) report, for two different samples of the Irish Cobbler variety, values of 135 and 150 μg., per 100 gm., respectively. Booher et al. ('39) found newly harvested potatoes of the latter variety to contain as much as 186 μg. per 100 gm. whereas old potatoes contained 147. In Nagel and Harris' ('43) study a value of 188 μg. per 100 gm. was reported. On the basis of data obtained by the Bureau of Home Economics, the Food and Nutrition Board of the National Research Council ('43) has assigned to the edible portion of the white potato an average thiamine content of 90 μg. per 100 gm., a value almost identical with that observed in this study, i. e., 89 μg. for the fourteen lots of potatoes.

Obviously the potato does not rank with pork products, glandular meats, whole cereal grains, or legumes as a source of thiamine. Because of its high calorie content it would be rated by the Council on Foods ('39) as only a fair source of the vitamin. This rating however scarcely gives adequate credit to a food which comprises such a large proportion of the daily food as does the potato. The quantities of this tuber suggested by the Bureau of Home Economics ('41) for a low-cost, a moderate cost, and a liberal, adequate diet for a very active man would, at the lowest average value for thiamine obtained in this study, i. e., 78 μg. per 100 gm., provide daily 0.38, 0.30 and 0.30 mg. thiamine or 16, 13, and 13%, respectively of the daily allowance (National Research Council, '43). Moreover the quantities of potatoes prescribed by the army (Howe, '42) would contribute 0.23 mg. or 11% of the total thiamine content of the rations. Certainly a food which could supply as much as one-sixth or one-ninth of the daily allowance of a nutrient must be considered a fairly significant source of that nutrient.

SUMMARY

The thiamine content of Green Mountain potatoes has been studied at various steps in the cooking and serving procedures. The percentage loss in thiamine incurred at each step has been computed on a dry-weight basis. Small losses resulted from soaking the potato prior to cooking and from holding the potato in a steam-oven for a period as long as 1½ hours. The greatest loss, 14.1% of the original value for thiamine, occurred during the steam-cooking process. The mashing of the potato did not appear to be destructive of the thiamine. The over-all loss in thiamine exclusive of that during soaking approximated 20%.

ACKNOWLEDGMENT

The authors are grateful to Mr. Walter Johnson, manager of the College Dining Hall and to his staff for their cooperation, and to Dr. Julia O. Holmes for help in the preparation of this manuscript.

LITERATURE CITED

AUGHEY, E., AND E. P. DANIEL 1940 Effect of cooking upon the thiamine content of foods. J. Nutrition, vol. 19, p. 285.

BOOHER, L. E., AND E. R. HARTZLER 1939 The vitamin B_1 content of foods in terms of crystalline thiamine, U. S. Dept. Agric. Tech. Bul. 707, 20 pp.

BOOHER, L. E., E. R. HARTZLER AND E. M. HEWSTON 1942 A compilation of the vitamin values of foods in relation to processing and other variants. U. S. Dept. of Agric. Circular 638, 244 pp.

BUREAU OF HOME ECONOMICS 1941 Planning diets by the new yardstick of good nutrition. U. S. Dept. of Agric. 14 pp

COUNCIL ON FOODS 1939 Accepted Foods and Their Nutritional Significance. Am. Med. Assn., Chicago, 492 pp.

FOOD AND NUTRITION BOARD 1943 Recommended dietary allowances. National Research Council, Reprint and Circular Series, no. 115.

HELLER, C. A., C. M. MCCAY AND C. B. LYON 1943 Losses of vitamins in large-scale cookery. J. Nutrition, vol. 26, p. 377.

HENNESSY, D J., AND L. R. CERECEDO 1939 The determination of free and phosphorylated thiamine by a modified thiochrome assay. J. Am. Chem. Soc., vol. 61, p. 179.

HOWE, P. E. 1942 Nutritional aspects of feeding an army. J. Am. Med. Assn., vol. 120, p. 93.

LANE, R L., E. JOHNSON AND R. R. WILLIAMS 1942 Studies of the average American diet I. Thiamine content. J. Nutrition, vol. 23, p. 613.

MOYER, J. C., AND D. K. TRESSLER 1942 Determination of thiamine in vegetables. Ind. and Eng. Chem., vol. 14, p. 788.

NAGEL, A. H., AND R. S. HARRIS 1943 Effect of restaurant cooking and service on vitamin content of foods. J. Am. Diet Assn., vol. 19, p. 23.

STUDENT 1925 New tables for testing the significance of observations. Metron, vol. 5, p. 105.

WIEGAND, J. A. 1938 Aneurinbestimmung in Getreidearten und Kartoffeln. Arch. Neerland, des Sci. Exact et Nat., Ser III. C. Arch. Neerland, de Physiol. d'Homme et Anim., vol. 23, p. 331.

REQUIREMENT OF TRYPTOPHANE BY THE CHICK

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ONE FIGURE

(Received for publication June 12, 1944)

Previous reports on the tryptophane requirement of the chick have been made (Klose, Stokstad and Almquist, '38; Almquist and Mecchi, '41). In the latter studies, acid-hydrolyzed casein supplemented with gelatin was used as the sole source of amino acids. Chick growth obtained with diets to which various levels of tryptophane had been added was used as an index to the tryptophane requirement, which was found to be approximately 0.5% of the diet. That this figure is probably too high was first suspected when analysis of casein by the method of Eckert ('43) yielded 1.2% tryptophane,[1] a value which would require feeding casein at 42% of the diet to provide sufficient tryptophane. It has already been shown that casein at the 20% level furnishes enough tryptophane for normal rate of gain (Almquist and Mecchi, '41). Accordingly, the tryptophane requirement was investigated further with diets not employing acid-hydrolyzed casein. The two types of diet used in the present study were (1) a mixture of 19 amino acids to which varying levels of natural and synthetic tryptophane were added, and (2) a zein-gelatin diet to which several supplementary amino acids were added.

EXPERIMENTAL

White Leghorn chicks were maintained from hatching time until 1 week of age on a practical rearing diet, at which time they were banded and weighed. Three days later they were weighed again, and on the bases of total weight, weight gain, and vigor, were segregated into groups of 2 to 5 chicks and housed in electrically heated, wire-floored battery brooders.

The diets were fed ad libitum for 12 days. The basal ration for the amino acid mixture diets was as follows: cellulose [2] 5, calcium gluconate 8, mineral mixture 3.24, crude soybean oil 5, sardine oil (400D-3000A

[1] We are indebted to Dr. F. H. Kratzer for permission to cite this result from his analyses.
[2] Cellu Flour.

per gram) to which 1% of mixed tocopherols [3] had been added 0.25, choline chloride 0.2, inositol 0.1, cholic acid 0.1, 2-methyl-1, 4-napthohydroquinone diacetate 0.001, thiamine 0.001, riboflavin 0.001, pyridoxine 0.001, nicotinic acid 0.003, calcium pantothenate *(dl)* 0.003, biotin (acid) [4] 0.00001 gm., with the amino acid mixture and glucose [5] to make 100 gm. Other members of the vitamin B-complex were provided by a concentrate prepared from solubilized liver which added only 4 mg. nitrogen to each 100 gm. diet. The mineral mixture furnished the following substances per 100 gm. of diet: tricalcium phosphate 2000, dipotassium phosphate 500, potassium chloride 300, and manganese 10, silicon 46, magnesium 48, aluminum 8, iron 14, copper 1, zinc 1, iodine 0.8, and cobalt 0.5 mg.

All of the amino acids in this diet were commercial products, and as far as possible, were from the same lot throughout the experiments. Data as to the levels used and the form in which the acids were added are summarized in table 1. The calculated amount of sodium bicarbon-

TABLE 1

Mixture of amino acids used in the basal diet.

AMINO ACID	FORM OF ACID	LEVEL AS FED	LEVEL OF NATURAL ISOMER
		%	%
Alanine	dl	1.0	0.5
Arginine	l (+) HCl	1.4	1.2
Aspartic acid	l (+)	2.0	2.0
Cystine	l (−)	0.4	0.4
Glutamic acid	l (+)	5.0	5.0
Glycine	—	1.8	1.8
Histidine	l (+) HCl H_2O	0.8	0.6
Hydroxyproline	l (−)	0.2	0.2
Isoleucine	dl	2.0	1.0
Leucine	l (−)	2.0	2.0
Lysine	l (+) HCl	1.4	1.1
Methionine	dl	1.0	0.5
Norleucine	dl	0.2	0.1
Phenylalanine	dl	1.0	0.5
Proline	l (−)	2.0	2.0
Serine	dl	0.4	0.2
Threonine	dl	3.0	1.5
Tryptophane		..	..
Tyrosine	l (−)	2.0	2.0
Valine	dl	2.0	1.0
	Total	29.6	23.6

[3] Natural mixed tocopherols, Type I (Distillation Products, Inc.).

[4] The biotin was kindly provided by Merck & Co., Inc., through the courtesy of Dr. J. C. Keresteszy.

[5] Cerelose.

ate was added to neutralize the hydrochlorides of the basic amino acids, and to provide sodium chloride, which was omitted from the mineral mixture generally used.

Except for the amino acid source, the zein basal diet was the same as the amino acid basal, with the nitrogen supplied by the following materials in %: zein 20, *l* (−)-cystine 0.3, *l* (+)-lysine 0.4, *dl*-methionine 0.2, *dl*-valine 1.0, *dl*-threonine 2.0 and gelatin (or fibrin) 10. Fibrin was used at a 10% level in one zein diet as a positive control for the completeness of the total protein source, as well as for the tryptophane, which it supplied at 0.32% of the diet.[1] When gelatin was used with zein, the diet contained less than 0.04% tryptophane. The supplements of natural and synthetic tryptophane [6] which were used were the same for all experiments. The specific rotation of the *l* (−) form was −31.2° at 20°C.[7]

RESULTS

The best rates of growth obtained in these studies were approximately 5% per day for a 10-day period, which is slightly less than that obtained with a methionine-supplemented soybean protein as the sole protein source for the chick (Grau and Almquist, '43). With the amino acid mixtures, however, some chicks did not eat the diets readily for the first day or two. Because of the variability of the early growth period with its consequent effect on the rate of gain, the efficiency of gain (gm. gain/gm. feed consumed) has been used, instead, as an index of the tryptophane requirement. Better agreement was obtained between experiments and between the two types of diets when the results were evaluated in this way. In both types of diets the maximal efficiency of gain was reached when 0.2 to 0.3% *l* (−)-tryptophane was present. This is approximately the amount furnished by 20% of intact casein, which is known to be an adequate source. The results of the various experiments are presented in table 2. The efficiency of gain has been plotted against the addition of tryptophane in figure 1. Curve A is based on average values of gain/feed for all experiments plotted against *l* (−)-tryptophane added. Consideration of curve B shows that the requirement for tryptophane in the *dl* form is twice that for the natural form, and indicates that the chick does not make appreciable use of the *d* (+) form for growth. From curve A it is estimated that the *l* (−)-tryptophane requirement of the chick is approximately 0.25% of the diet for maximal efficiency of utilization of

[6] Merck & Co., Inc.

[7] $[a]_D^{20}$ for pure *l* (−)-tryptophane in given as −31.5°. (Greenberg, '44).

TABLE 2

Relation of added natural and synthetic tryptophane to efficiency of feed utilization.

DIET NO.	NO. CHICKS PER GROUP	TYPE OF DIET	TRYPTOPHANE ADDED		GAIN PER GM. FEED CONSUMED
			l (−)	dl	
			%	%	*gm.*
1	2	Amino acid mixture	0	0	− 0.32
			0	0.1	− 0.07
			0.1	0	+ 0.08
			0	0.2	+ 0.08
			0.2	0	+ 0.33
2	4	Amino acid mixture	0.1	0	+ 0.17
			0	0.2	+ 0.06
			0.2	0	+ 0.29
			0	0.4	+ 0.27
			0.3	0	+ 0.30
			0	0.6	+ 0.31
			0.4	0	+ 0.31
			0	0.8	+ 0.30
3	5	Zein + gelatin	0	0	− 0.45
			0.1	0	+ 0.17
			0.2	0	+ 0.27
			0.3	0	+ 0.33
			0.6	0	+ 0.29
4	5	Zein + fibrin	0.32	0	+ 0.33

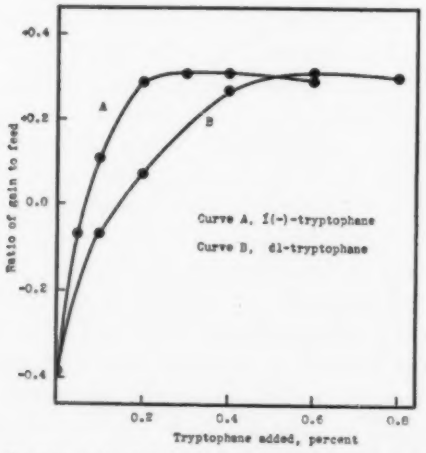

Fig. 1 The effect of the per cent of *l* (−)- and *dl*-tryptophane added to the diet on the ratio of chick weight gain to feed eaten.

the diet. Maximal rates of increase in body weight were also attained at approximately this level of *l* (—)-tryptophane.

Since the requirement previously suggested was approximately twice the value reported here, it seems possible that the isolated, natural tryptophane used in the former studies may have been largely racemized. The present results reveal a further difference between the amino acid requirements of the chick and of the rat, for the latter animal can apparently utilize both isomers of tryptophane for growth (Berg and Potgeister, '31–'32; du Vigneaud, Sealock, and Van Etten, '32; Berg, '36). In the mouse less rapid growth is obtained with the unnatural form than with the natural form (Totter and Berg, '39).

SUMMARY

Use of a diet containing a mixture of 20 amino acids or one containing zein and gelatin supplemented with some pure amino acids as the only protein source has shown *dl*-tryptophane to be only half as effective as *l* (—)-tryptophane in promoting growth and efficient food utilization in the chick. From the results obtained with these diets, the *l* (—)-tryptophane requirement is shown to be approximately 0.25% of the diet.

ACKNOWLEDGMENT

This study was facilitated by research grants from the Nutrition Foundation, Inc., and Swift and Co.

LITERATURE CITED

ALMQUIST, H. J., AND E. MECCHI 1941 Tryptophane requirement of the chick. Proc. Soc. Exp. Biol. and Med., vol. 48, p. 526.

BERG, C. P. 1936 Tryptophane metabolism IV. The influence of optical activity on the utilization of tryptophane for growth and for kynurenic acid production. J. Biol. Chem., vol. 104, p. 373.

BERG, C. P., AND M. POTGEISTER 1931–32 Tryptophane metabolism. II. The growth-promoting ability of *dl*-tryptophane. J. Biol. Chem., vol. 94, p. 661.

DU VIGNEAUD, V., R. R. SEALOCK AND C. VAN ETTEN 1932 Availability of *d*-tryptophane and its acetyl derivative to the animal body. J. Biol. Chem., vol. 98, p. 565.

GREENBERG, D. M. 1944 "Optical properties of amino acids and proteins." *In* The chemistry of the amino acids and proteins, p. 1177. C. L. A. Schmidt, editor. Charles C. Thomas, publisher, Springfield, Ill.

KLOSE, A. A., E. L. R. STOKSTAD AND H. J. ALMQUIST 1938 The essential nature of arginine in the diet of the chick. J. Biol. Chem., vol. 123, p. 691.

TOTTER, J. R., AND C. P. BERG 1939 The influence of optical isomerism on the utilization of tryptophane, histidine and lysine for growth in the mouse. J. Biol. Chem., vol. 127, p. 375.

THE FRACTIONATION OF PHOSPHORUS COMPOUNDS IN CERTAIN VEGETABLES[1]

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(Received for publication June 9, 1944)

Phosphorus compounds in plant and animal tissues and fluids, so far as known, are formed from ortho-phosphoric acid. The capacity for the characteristic reactions is dependent upon the three hydroxyl groups. They not only react with other compounds but they may also react with other molecules of phosphoric acid giving rise to di- or tri-phosphoric acid compounds such as one finds in the nucleotide adenosine. Since phosphoric acid is capable of forming many more or less distinct groups of substances with many functions (James and Arney, '39; Peters and Van Slyke, '31) different concentrations of some fractions should be found in plants having different metabolic patterns. Such information may be of value in understanding further the role of the element once referred to as "phosphorus mirabilis" (Thorpe, '28). Furthermore, since vegetables may furnish an increased proportion of our phosphorus requirements it is desirable to determine whether or not the organic phosphorus exists in available forms in certain foods (Lowe and Steenbock, '36; Lowe, Steenbock and Krieger, '39; McCance and Widdowson, '35; Yang and Dju, '40).

This paper therefore presents a quantitative fractionation of the phosphorus compounds of eleven common vegetables into the following groups: inorganic, organic, resistant esters, phosphoprotein and phospholipid phosphorus, and phytin.

EXPERIMENTAL

The values reported for each vegetable represent the average of duplicate or triplicate determinations on one sample which was prepared from several kilograms of representative fresh material.

The scheme of separation was similar to that used by Arney ('39). All manipulations were checked for the recovery of phosphorus by the use of solutions of known phosphorus content. All phosphorus determinations were made colorimetrically by the Truog and Meyer method ('29). Total phosphorus was brought into solution by wet ashing

[1] Contribution no. 529 of the Massachusetts Agricultural Experiment Station.

with sulphuric and nitric acids. Resistant esters and inorganic phosphorus were determined on clarified trichloroacetic acid extracts. Extractions with trichloroacetic acid made over periods of 30, 60 and 90 minutes indicated that an end point was reached in 30 minutes. The original solutions were too colored to use directly, hence all phosphorus compounds were precipitated by a procedure similar to that of Needham, Needham, Baldwin and Yudkin ('32). The precipitate was dissolved in an acid solution of pH 1. The inorganic fraction was determined directly on this solution, while a total phosphorus determination was also made by wet ashing the extract, in order to obtain the resistant esters by difference. Phospholipid and phosphoprotein phosphorus constituted the difference between the total phosphorus and the acid soluble phosphorus. Phytin phosphorus was determined by the McCance and Widdowson method ('35).

The results of the investigation are shown in table 1.

TABLE 1

The percentage content of phosphorus on a dry matter basis in various forms in certain vegetables and their percentage distribution.

VEGETABLE	TOTAL	INORGANIC	ORGANIC	RESISTANT ESTERS	PHOSPHOLIPIDS AND PHOSPHOPROTEINS
Asparagus, Asparagus officinalis L.	0.699	0.359 (51.4)[1]	0.340 (48.6)	0.184 (26.3)	0.156 (22.3)
Cabbage, Brassica oleracea L.	0.414	0.256 (61.8)	0.158 (38.2)	0.080 (19.3)	0.078 (18.9)
Carrot, Daucus carota L.	0.350	0.247 (70.6)	0.103 (29.4)	0.059 (16.9)	0.044 (12.5)
Dandelion, Taraxacum officinale W.	0.439	0.257 (58.5)	0.182 (41.5)	0.068 (15.5)	0.114 (26.0)
Eggplant, Solanum melongena L.	0.271	0.196 (72.3)	0.075 (27.7)	0.040 (14.8)	0.035 (12.9)
Kale, Brassica oleracea L.	0.437	0.256 (58.6)	0.181 (41.4)	0.062 (14.2)	0.119 (27.2)
Spinach, Spinacia oleracea L.	0.873	0.568 (65.1)	0.305 (34.9)	0.190 (21.8)	0.115 (13.1)
String beans, Phaseolus vulgaris L.	0.505	0.357 (70.7)	0.148 (29.3)	0.044 (8.7)	0.104 (20.6)
Squash, Cucurbita maxima D.	0.423	0.213 (50.4)	0.210 (49.6)	0.029 (6.9)	0.181 (42.7)
Turnip, Brassica rapa L.	0.543	0.335 (61.7)	0.208 (38.3)	0.120 (22.1)	0.088 (16.2)
Onions, Allium cepa L.	0.291	0.185 (63.6)	0.106 (36.4)	0.087 (29.9)	0.019 (6.5)

[1] The figures in parentheses indicate the percentage of the total phosphorus which each fraction represents.

Inorganic phosphorus ranged from about 50 to over 72% of the total. Resistant esters averaged slightly less than the phospholipid and phosphoprotein phosphorus; the former varied from about 7 to 30%, the latter from 6.5 to 42.7%. Phytin was absent in all cases.

SUMMARY

From these data one may conclude that the bulk of the phosphorus is in the inorganic form; that the organic fraction may be composed of widely varying amounts of resistant esters, and the phosphoproteins and phospholipids; and that there are no unavailable forms of phosphorus present in these vegetables.

LITERATURE CITED

ARNEY, S. E. 1939 CXXXI Phosphate fractions in barley seedlings. Biochem. J., vol. 33, p. 1078.

JAMES, W. O., AND S. E. ARNEY 1939 Phosphorylation and respiration in barley. New Phytologist, vol. 38, p. 340.

LOWE, J. T., AND H. STEENBOCK 1936 Cereals and Rickets VIII. The hydrolysis of phytin in the intestine. Biochem. J., vol. 30, p. 1991.

LOWE, J. T., H. STEENBOCK AND C. H. KRIEGER 1939 Cereals and Rickets IX. The availability of phytin phosphorus to the chick. Poultry Sci., vol. 18, p. 40.

MCCANCE, R. A., AND E. M. WIDDOWSON 1935 CCCXX. Phytin in human nutrition. Biochem. J., vol. 29, p. 2694.

NEEDHAM, DOROTHY M., J. NEEDHAM, E. BALDWIN AND J. YUDKIN 1932 A comparative study of the phosphagens with some remarks on the origin of vertebrates. Proc. Roy. Soc. (London) B., vol. 110, p. 260.

PETERS, P., AND D. D. VAN SLYKE 1931 Quantitative Clinical Chemistry, vol. 1, p. 1100. The Williams and Wilkins Co., Baltimore.

THORPE, T. E. 1928 A comprehensive treatise on inorganic and theoretical chemistry. J. W. Mellor, vol. 8, p. 729.

TRUOG, E., AND A. H. MEYER 1929 Improvements in the Deniges colorimetric method for phosphorus and arsenic. Ind. Eng. Chem. Anal. Ed., vol. 1, p. 136.

YANG, E. F., AND M. Y. DJU 1940 Total and phytic acid phosphorus in foods. Chinese J. Physiol., vol. 14, p. 473; Chem. Abstracts, vol. 34, p. 4815.

EFFECTS OF VARIATIONS IN DIETARY PROTEIN ON THE PHYSICAL WELL BEING OF MEN DOING MANUAL WORK[1]

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(Received for publication May 27, 1944)

INTRODUCTION

The question of the effects of low and high protein diets has become an immediately practical one, not only because of the relative scarcity of high protein foods but also because of the greater difficulty in shipping them long distances. Previous work has shown clearly what was the minimum protein intake necessary to maintain nitrogen equilibrium (Reviews — Lusk, '31 and Terroine, '36) and that low levels are compatible with health in sedentary men. Although frequent statements are to be found that higher intakes are necessary for hard work, almost none of these is supported by experimental proof. In fact the most extensive experimental work, that of Chittenden ('04), reported increased vigor for hard working men when the protein intake was lowered to 50–60 gm.

In order to make the results of this work useful for direct practical application, the aim was to utilize normal young men in a natural environment carrying on their usual regime of work. For the same reason the diets were planned to include only items to be found in normal diets, and thus to avoid synthetic or laboratory foods which would not be encountered in practical dietary experience. The plan in one group of subjects was to restrict protein foods as much as possible within the above limitations; in another group of subjects to give the greatest possible excess of high protein foods. In a third group the individuals were to continue on their usual diet.

The conclusions from this work must be limited to results appearing within the time limit of the tests, namely 8 weeks of the modified diet.

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

Therefore, the practical implications must likewise be limited to apply to emergency modifications of diet lasting up to 2 months.

METHODS

The subjects were twenty-four volunteers from the personnel of a Civilian Public Service Camp, who continued on the work regime of the camp and ate their meals at special tables in the regular mess hall. At the start each subject was given a thorough physical examination, clinical laboratory examination of blood and urine and blood chemical estimation of NPN, protein and A/G Ratio. NPN was estimated by the method of Daly ('33); serum protein and urinary nitrogen by the Micro Kjeldahl method of Ma and Zuazaga ('42); and serum protein fractionation by the method of Howe ('21). Throughout the study frequent check-up physical examinations were held and the estimations of blood NPN and protein were repeated weekly along with a measurement of the nitrogen in a 24-hour specimen of urine. Assessment of physical fitness each week was by means of the so-called "Pack Test." In this test the subject, wearing a rucksack weighted to approximately ⅓ his body weight stepped up onto a 16-inch platform and back down onto the floor once each 2 seconds (handrails at shoulder height were used as an aid). The stepping exercise was terminated at 5 minutes if the subject could carry on that long; after stopping, the pulse was counted for three periods: 1 to 1½ min., 2 to 2½ min. and 4 to 4½ min. of the recovery period. From these data a numerical score was calculated as:

$$\frac{\text{duration exercise in seconds}}{2 \times (\text{Sum of pulse beats counted in the three periods})}$$

This test had been found previously to correlate with physical ability in athletes and to parallel improvements with training and deterioration accompanying proven inadequate diets. In order to have an additional index of recuperative powers, the test was administered twice on each occasion with only 15 minutes rest between the two.

The dietary test program consisted of three periods — 2 weeks preliminary control, 8 weeks of modified diet and 2 weeks of final control. The twenty-four subjects were divided in three groups of eight, each group including a rough cross section of the whole in respect to occupation and physical fitness. All groups ate the regular camp diet for the first 2 weeks and one of the groups of eight continued thus throughout. A second group, called the restricted protein group, changed their diet at the end of the first control period to one which allowed no meat,

cheese, eggs, nuts, legumes and only up to ½ cup of milk daily. Bread was the only other food allowed the restricted protein group which contained animal protein (milk); this was limited in amount to this group so that not more than 2½ gm. of milk protein was furnished daily in this form and not more than 5 gm. of animal protein in the entire daily diet (the other 2½ gm. or less in top milk on cereal). Unlimited amounts of low protein foods were furnished this group and they, like the other groups, were urged to fully satisfy their appetites. During the final control period this group was given a high protein diet.

The third group, called the high protein group, was furnished with large amounts of high protein foods and allowed only restricted amounts of low protein foods.

All groups were given daily 5 gm. of yeast extract [2] fortified with additional riboflavin to forestall any possibility of vitamin B deficiency. As will be noted from the above description, the dictates of taste were followed in so far as possible in this type of experiment. For measurements of the diet each mess table was furnished with a diet scales and standard ladles. All articles of food were furnished in standard portions of known weight or else the subjects weighed the portion eaten. Each subject had a special record book with a page for each day in which he noted the amounts of each article of food eaten during the day on one side of the sheet and the day's activities hour by hour on the other side. These books were kept in a rack in the dining room. The proper analytic figures for protein, fat and calories were entered in these books periodically by the dietitian staff. All items in the diet known to contain significant amounts of protein were analyzed at least once for protein during the experiments. Those subject to variation (e.g., bread, cereals) in cooking were analyzed each time they were on the menu. Our own analytic figures were supplemented with those of Bowes and Church ('40) for fat and calories and for the traces of protein in low protein foods. Whenever possible the manufacturer or baker was consulted to determine ingredients.

The work of the subjects included office work, kitchen work, laundry work, farming and forestry work (including work on trails, clearing forest ground and repair of roads). The daily caloric expenditure, depending on the subject's job ranged from 2400 to 5000 cal. with an average of approximately 3300.

[2] Standard Brands Co., Type 3. This amount of fortified yeast contained approximately 5 mg. thiamine, 1.5 mg. riboflavin and 10 mg. niacin.

RESULTS

I. Restricted protein group

The averages of daily protein intakes in this group of eight are shown in table 1 along with the caloric intakes, subjects' weights and their usual occupations. It will be seen that even with the severe restriction of high protein foods the daily protein intake was rarely below 50 gm. and averaged 53 gm. When the caloric intake was above 4000 cal. the protein was usually nearer 60 gm. Potato, bread and other cereal products were the chief sources of this protein; less than 5 gm. was of animal origin. Two subjects of this group terminated their experiment early, one because of an acute attack of recurrent appendicitis, the other because of transfer to another project.

The 24-hour urinary excretions of nitrogen were measured once a week and the results, expressed as grams of equivalent protein, are included in table 1. Since these figures are calculated from samples taken only once weekly whereas the figures for intake were obtained daily, exact agreement should not be expected. However, it can be seen that the urinary nitrogen figures furnish a rough check on the calculated intake, if the usual assumption of 1 gm. of stool nitrogen is made. If anything, the intake figures tend to be a little larger than the corrected urinary figure so it may be safely stated that the calculated mean daily intake figure of 53 gm. is perhaps too high.

In general all the subjects in this group believed they were getting enough food. It was their observation, however, especially those doing the heaviest work, that they became unusually hungry and felt a little weak late in the morning and late in the afternoon. There was an average weight loss during the 8-week period of 0.9 kg., a good part of which could be logically explained by the fact that several of the group started on a regime of much heavier outdoor work than was customary to them. A weight loss of similar magnitude was found in the group on the normal diet. One man who lost 2.8 kg. was obese and rather "flabby" at the start.

Aside from the experiences of late morning and afternoon hunger mentioned above, none of the subjects complained of unusual symptoms. They were able to carry on the work routine; no complaints by their work supervisors were ever made. The pack test data confirms the impression that they showed no deterioration. Table 2 summarizes these results on this group and those on the normal and high protein diets. In spite of some irregularities it will be seen that both groups improved slightly; a few individuals strikingly, undoubtedly

TABLE 1

Summary of caloric intakes, protein intakes and urinary nitrogen excretions expressed as "equivalent protein".

Diet	Individual	Occupation	Body weight	Preliminary control period (daily)			Experimental period: 1st four weeks (daily)			Experimental period: 2nd four weeks (daily)			Final control period (daily)		
				Caloric intake [1]	Protein intake [1]	Protein metabolism [2]	Caloric intake [1]	Protein intake [1]	Protein metabolism [2]	Caloric intake [1]	Protein intake [1]	Protein metabolism [2]	Caloric intake [1]	Protein intake [1]	Protein metabolism [2]
			kg.		*gm.*	*gm.*		*gm.*	*gm.*		*gm.*	*gm.*		*gm.*	*gm.*
Normal diet	Co	Forestry	71.1	2830	100	..	3870	125	102	....	..	..	....	..	..
	H	Truckdriving	72.0	3140	110	108	3640	115	81	3240	108	91	3350	114	101
	K	Camp maintenance	75.6	2720	94	72	2830	101	80	2920	107	99	2960	97	77
	Lar	Kitchen	77.0	3080	123	..	2750	102	68	3460	131	71	2790	97	79
	M	Carpentry	72.3	3550	128	102	3460	107	92	3140	105	94	2970	92	104
	N	Kitchen	72.8	2460	81	85	2060	71	63	2280	76	61	....	..	..
	Su	Office	68.3	3590	126	104	3620	123	98	3130	108	80	3060	92	67
	Tr	Carpentry	73.8	3040	101	79	3150	106	75	3380	102	85	3190	95	54
		Average	72.9	3050	108	92	3170	106	82	3080	105	83	3050	98	80
Restricted protein	B	Truckdriving	85.0	3090	110	88	3300	55	42	3230	57	41	4440	189	128
	F	Kitchen	79.7	3240	103	100	3750	57	45	3800	53	40	4110	185	141
	G	Road repair	59.2	2770	75	58	3020	52	41	3230	52	36	3810	135	114
	J	Office	87.7	2900	83	67	3140	56	54	....	..	..	....	..	..
	R	Farming	69.7	3600	125	127	3750	57	66	4300	59	47	5600	274	207
	Sm	Road construction	85.9	3490	117	108	3470	57	51	....	..	..	....	..	..
	Ste	Kitchen	74.9	2800	92	96	2260	39	43	2950	43	34	3710	184	73
	Sto	Forestry	63.3	3500	122	99	3170	48	44	3350	50	41	4160	198	131
		Average	76.9	3180	103	93	3230	53	48	3480	53	40	4310	194	132
High protein	A	Office	69.6	2080	78	61	2330	96	83	2380	110	88	2340	88	69
	C	Farming	68.9	3660	142	120	3980	190	156	3980	189	179	3150	125	117
	D	Kitchen	107.0	2460	103	98	3290	139	128	3590	182	144	2750	100	123
	Li	Office	83.2	2980	97	75	3660	153	122	3770	159	115	3150	104	103
	Lo	Forestry	80.0	3810	126	112	4440	193	164	3810	172	163	3150	112	107
	Sa	Forestry	84.3	3410	114	..	3950	174	161	5010	254	153	3290	137	124
	Tu	Office	72.4	2980	108	104	3270	151	132	3820	176	135	2380	82	68
	W	Laundry	62.6	3240	114	98	4430	185	156	4090	175	159	3060	106	98
		Average	78.6	3080	110	95	3670	160	138	3810	177	142	2910	107	101

[1] Average values calculated from weights of food eaten and analytic figures (all days calculated).

[2] Average values from analysis for nitrogen of 24-hour urine, collected only once weekly (gm. nitrogen x 6.25 to obtain figure in gm. protein for comparison with intake).

due chiefly to the physical training from their active work regime. The irregularities of scores are in some cases due to changes in motivation on different days; in other cases to local disabilities arising from minor injuries. The relative scores on the two consecutive tests on the same day did not yield any additional information and are not reported in detail. It was an unexplained fact that the relationship of the second to the first score was largely characteristic of the individual: many dropped off slightly every time on the second score, a few markedly, while a few actually improved in every instance.

TABLE 2

Physical fitness as indicated by scores in "pack test".

DIET	SUBJECT	PRELIMINARY CONTROL PERIOD: WEEK		EXPERIMENTAL PERIOD: WEEK								FINAL CONTROL PERIOD: WEEK	
		1st	2nd	1st	2nd	3rd	4th	5th	6th	7th	8th	1st	2nd
Normal diet	Co	71	..	81	78	72	..	..	..	..	..	..	..
	H	105	..	92	99	110	110	102	103	113	102	108	106
	K	57	50	55	61	62	58	67	65	58	68	65	52
	Lar	31	..	52	40	37	81	79	88	86	68	89	69
	M	43	78	85	86	96	102	96	87	87	88	106	99
	N	70	58	53	47	39	31	48	53	52	61	..	..
	Su	46	82	77	81	77	73	83	85	86	73	86	91
	Tr	..	..	62	67	74	85	88	86	86	85	89	92
Average increase [1]					−6	−4	+1	+5	+6	+6	+3	+15	+9
Restricted protein	B	26	23	27	34	37	34	38	42	48	85	54	79
	F	68	77	84	80	77	82	95	91	95	84	89	87
	G	48	75	85	85	96	77	82	88	90	84	83	..
	J	30	45	53	48	30	44	..	..	..	..	..	..
	R	81	82	82	..	..	82	73	80	82	..	82	79
	Sm	56	58	79	..	87	85	90	..	..	..	..	..
	Ste	67	87	88	95	89	103	98	107	88	90	99	86
	Sto	92	93	111	102	97	93	96	101	111	101	106	98
Average increase [1]					−1	−2	−1	+2	+6	+6	+10	+6	+7
High protein	A	71	69	73	68	77	70	84	83	79	84	83	80
	C	84	87	92	90	90	92	96	92	94	85	88	91
	D	32	37	52	35	48	47	54	62	55	38	50	53
	Li	69	..	83	94	87	80	84	78	86	79	93	87
	Lo	97	87	94	89	93	98	85	82	91	87	89	80
	Sa	109	88	..	100	..	107	104	108	111	108	105	134
	Tu	77	81	83	80	79	83	83	83	87	87	79	83
	W	25	..	25	23	33	27	25	43	37	34	35	46
Average increase [1]					−4	+2	0	0	+3	+3	−2	+1	+5

[1] These figures obtained by setting for each individual a standard score equal to the highest of his first three scores on the table, by calculating for each later score the difference from the standard, and then by averaging the individual differences for each week.

The blood chemical and hematological findings were completely negative. There was no trend toward a lowering of either albumin or total protein; the red blood count and hemoglobin remained essentially unchanged.[3]

In the case of this group the final control period had a special significance since the protein in the diet was raised to a high level at this time. No change in physical performance or chemical findings was evident aside from the increased amount of nitrogen in the urine. The subjective effect of the change was conditioned by the preferences of the men; two men, habitual lovers of meat, were much happier on the high protein diet; two men found the high protein diet actually distasteful, the remainder had no strong feelings one way or the other.

II. High protein group

Again in this group the changes due to alterations in diet were practically non-existent. The weekly averages of daily protein intake ranged from 157 to 192 gm. compared to 95 to 113 in the normal group (see table 1 for 4-week averages). The average in the high protein group was kept down by one subject, a sedentary, rather hypersensitive individual to whom "heavy food" was distasteful.

The less active men in this group tended to feel overfull and sleepy after meals. Not all this effect was necessarily due to the high protein per se. Among the available common foods high protein is associated with considerable fat. As a result the total caloric intake in this group tended to be higher than necessary. This is clearly shown in the weight figures which show an average gain of 1.0 kg. and a gain in one individual of over 5 kg. No other unusual symptoms were experienced.

The physical fitness tests showed a tendency to gradual improvement (table 2) as in the other groups, which is likewise explainable by training.

The urinary excretion of nitrogen expressed as equivalent protein in table 1 gives figures of 138 gm. and 142 gm. daily for the two 4-week periods. Although not exactly to be compared with the intake figures because of the less frequent measurements (see under Restricted Protein Group above), these figures confirm the high protein metabolism of this group but would require the assumption of rather high values of stool nitrogen (about 6 gm.) to be brought in line with the calculated intakes.

[3] Details of these measurements are omitted for the sake of brevity but will be furnished by the authors on request.

. The only change in chemical findings [3] was a tendency to slightly higher NPN which is probably not very significant.

DISCUSSION

These studies fail to confirm Chittenden's conclusions that a restricted protein diet improves physical well being. His experiments had the advantage of being longer in duration but the disadvantage of having no parallel control subjects on their usual diet. His conclusion that there is no impairment of health by such a diet is completely supported.

It is apparent from these studies that to choose a low protein diet from commercial articles of diet is difficult, provided caloric balance is maintained. When sufficient calories are given for hard physical labor, a fairly respectable level of protein is automatically included.

Likewise it was found difficult to increase the protein level above 150 gm. using standard items of diet. To push beyond this without giving excessive calories, defatted foods such as buttermilk or skim milk would be necessary.

The conclusions from this study should not be extended beyond the conditions investigated. The protein needs during growth, illness and lactation are wholly outside the scope of this work. It is apparent only that for 2 months with normal men (such as laborers or soldiers) rather extreme variations in protein intake were without measurable effect either beneficial or harmful. The practical implications are that under emergency conditions a diet supplying about 50 gm. of protein, chiefly from potatoes and grain products, is not incompatible with the health of physically active young men.

SUMMARY AND CONCLUSIONS

1. Within 2 months no measurable influence either deleterious or beneficial could be observed on the physical vigor or efficiency of eight healthy young men subsisting on a diet adequate in calories but restricted in protein. The daily protein intake averaged 50 to 55 gm., very little of which was of animal origin.

2. Similarly no beneficial or harmful effect could be observed in 2 months on eight men subsisting on a diet providing 160 gm. or more of protein, mostly first class.

ACKNOWLEDGMENTS

This study was made possible only by the complete cooperation of the authorities and personnel of C. P. S. Camp no. 32 where it was held. The authorities concerned include both those of the Selective Service System and of the American Friends Service Committee. The camp personnel to whom we are indebted include not only the volunteer subjects, who added the hardships of the diet to a busy schedule, but also to various members of the maintenance personnel who assisted in many tasks connected with the experiments.

We are indebted to Dr. F. J. Stare and Dr. D. M. Hegsted of the Nutrition Department, Harvard Medical School for helpful suggestions on the choice and preparation of diets and for their loan of dietitians.

Various members of the U. S. Forestry Service sssisted us throughout by various courtesies.

We acknowledge substantial financial assistance from the Nutrition Foundation, Inc.

LITERATURE CITED

Bowes, A. de P., and C. F. Church 1940 Food values of portions commonly used. College Offset Press, Philadelphia, 31 pp.

Chittenden, R. H. 1904 Physiological economy in nutrition. Frederick A. Stokes Co., New York.

Daly, C. A. 1933 The determination of non-protein nitrogen with special reference to the Koch-McMeekin method. J. Lab. and Clin. Med., vol. 18, p. 1279.

Howe, P. E. 1921 The use of sodium sulfate as the globulin precipitant in the determination of protein in the blood. J. Biol. Chem., vol. 49, p. 93.

Lusk, G. 1931 The science of nutrition. W. B. Saunders Co., Philadelphia.

Ma, T. S., and G. Zuazaga 1942 Micro Kjeldahl determination of nitrogen. J. Ind. and Eng. Chem., vol. 14, p. 280.

Terroine, E. F. 1936 The protein component in the human diet. Quarterly Bulletin Health Organization League of Nations, vol. 5, p. 427.

ABSENCE OF NERVE DEGENERATION IN CHRONIC THIAMINE DEFICIENCY IN PIGS [1]

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ONE FIGURE

(Received for publication April 18, 1944)

In earlier reports (Wintrobe, Stein, Miller, Follis, Najjar, and Humphreys, '42 b; Follis, Miller, Wintrobe and Stein, '43) we described our failure to produce degenerative changes in the nervous system of pigs by inducing thiamine deficiency. This observation was contrary to the rather generally held view that thiamine is "the antineuritic vitamin." Since our studies at that time were limited to nine pigs, this report concerning observations in ten additional animals is recorded. In the accompanying table the pertinent data for all nineteen animals are summarized.

The care and management of the animals was similar to that followed in earlier studies (Wintrobe, Miller, Follis, Stein, Mushatt and Humphreys, '42 a) and need not be described in detail again. The basal diet consisted of casein 26.1, sucrose 57.7, lard 11.0, and a complete salt mixture 5.2. In the present series there were four pigs which received only crystalline vitamins as the source of the "B-complex." Six animals were given autoclaved yeast. This was done because in most of the experiments reported hitherto, diets supplemented by autoclaved yeast have been used, the purpose of autoclaving being to destroy thiamine. Since we have observed neurological lesions in pigs receiving a diet deficient in pyridoxine or pantothenic acid (Wintrobe, Miller, Follis, Stein, Mushatt and Humphreys, 42 a), the question arose whether the pyridoxine or, more probably, the pantothenic acid content of yeast might also be lowered by autoclaving. Two of our animals receiving autoclaved yeast were given pyridoxine and calcium pantothenate as well.

[1] Aided by grants from the Rockefeller Foundation, Parke-Davis and Company, the Upjohn Company, and the Fleischmann Laboratories, and carried out in cooperation with the Bureau of Animal Industry, U. S. Department of Agriculture.

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The yeast used was a brewers' yeast available commercially.[3] "Dry" autoclaved yeast was prepared by autoclaving for 6 hours at 20 pounds pressure. "Alkaline" autoclaved yeast was prepared by mixing yeast with 0.1 N sodium hydroxide and autoclaving as above, neutralization being carried out afterwards. These two methods of autoclaving were employed because each method has been used by various workers (Swank, '40; Bessey, '42). The pantothenic acid content of the untreated yeast, as measured by microbiological assay (Strong, Feeney and Earle, '41) was 72 μg. per gm. That of the "dry" autoclaved yeast was 14 μg. while the "alkaline" autoclaved material contained 46 μg.

In none of the animals was abnormal gait observed during life and in none were histologic changes discovered in the nervous system in spite of painstaking study by accepted methods. This is in agreement with the observations of Ellis and Madsen ('44). There can be no question that thiamine deficiency was produced in these animals for loss of appetite, vomiting and impairment of growth were observed in all, these symptoms could be relieved by giving thiamine, and death occurred as the result of cardiac failure in all except those animals which were sacrificed in a condition of less severe thiamine deficiency. Pronounced electrocardiographic changes were observed in these pigs (Wintrobe, Alcayaga, Humphreys and Follis, '43 a) as well as extensive lesions in the myocardium and evidence of congestive cardiac failure (Follis, Miller, Wintrobe and Stein, '43).

In order to imitate the conditions likely to be encountered in humans, the thiamine deficiency was only partial. This procedure also achieved another purpose. It has been argued that nerve degeneration is not to be expected in animals acutely deficient in thiamine and it has been implied that changes in the nervous system will be observed if the deficiency is of sufficiently long standing. It will be noted that many of our animals were maintained in a chronically deficient state for 7 to 8½ months, one even longer. The growth curves shown in figure 1 and those shown in an earlier report (Wintrobe, Stein, Miller, Follis, Najjar and Humphreys, '42 b), indicate the "chronicity" of these experiments. Once the experiment was commenced, care was taken at all times to maintain the thiamine supplement at a level well below that required by the pigs. This level was so low at times that some of the pigs died sooner than we would have wished (see table 1).

[3] Mead Johnson and Company, Evansville, Ind.

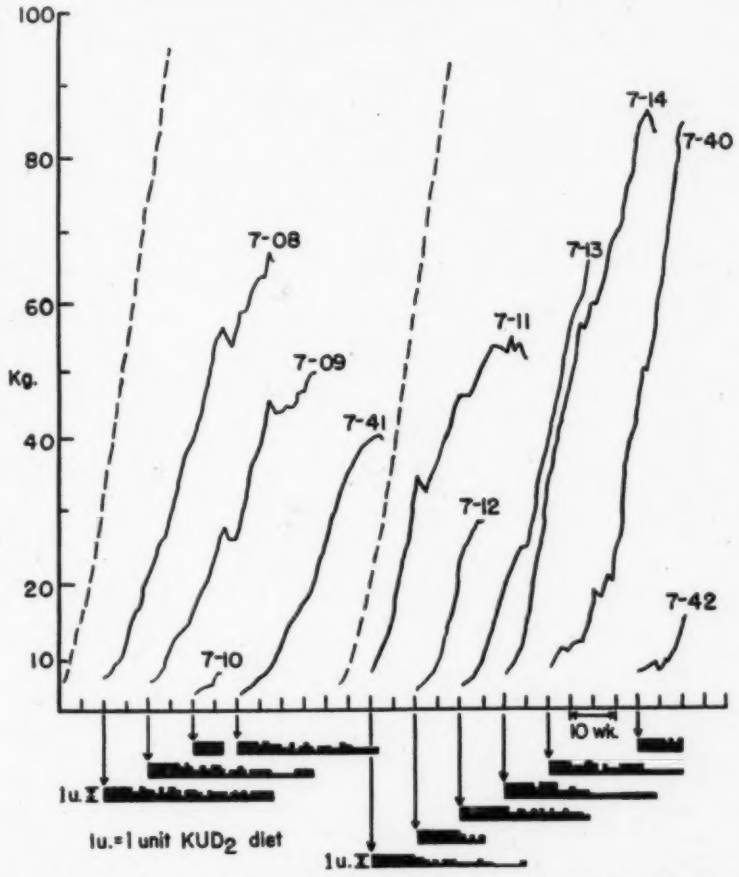

Fig. 1 Growth curves and food consumption of animals receiving thiamine-deficient diets. One unit of the diet furnished approximately 152 cal. per kilogram body weight.

The broken curve appearing at intervals represents the growth of pigs at the Beltsville Research Center given a mixed diet and fed and handled according to record of performance procedure.

DISCUSSION

It does not necessarily follow as a result of these studies that nerve degeneration in man is never the result of thiamine deficiency. There has been ample demonstration of the fact that the manifestations of vitamin deficiency, as well as the requirements for the various vitamins, are not the same in all species. It is to be noted, however, that when nerve degeneration has developed in man in association with nutritional deficiency, the deficiency has been multiple and there has

TABLE 1

Summary of data for 19 thiamine-deficient pigs.

DIETARY SUPPLEMENTS	PIG NO.	AGE EXPT. ENDED	DURATION OF DEFICIENCY	AMOUNT OF THIAMINE GIVEN Average and range	CAUSE OF DEATH	NERVOUS SYSTEM
		days	*days*	*μg./kg. body wt.*		
RNB$_s$ChP [1]	6-53 [2]	246	156	14 (0–35)	Cardiac failure	Normal
	6-67 [2]	171	83	0	Cardiac failure	Normal
	6-77 [2]	334	246	14 (0–35)	Cardiac failure	Normal
	6-90 [2]	134	57	10 (10)	Cardiac failure	Normal
	6-91 [2]	107	37	10 (10)	Cardiac failure	Normal
	6-92 [2]	210	141	20 (10–60)	Sacrificed	Normal
RNB$_s$ChPIp	7-08	296	258	17 (3–65)	Cardiac failure	Normal
	7-09	290	254	15 (3–65)	Cardiac failure	Normal
	7-10	76	41	28 (15–65)	Cardiac failure	Normal
	7-41	238	202	17 (10–20)	Cardiac failure	Normal
Yeast, autoclaved (dry) [3]	7-11	273	231	0	Cardiac failure	Normal
Yeast, autoclaved (dry) [3]	7-40	244	206	0	Sacrificed	Normal
Yeast, auto. (dry) B$_s$P [4]	7-14	280	238	0	Sacrificed	Normal
Yeast, autoclaved (alk.)	7-12	147	103	(See footnote 4)	Cardiac failure	Normal
Yeast, autoclaved (alk.)	7-42	101	67	(See footnote 4)	Cardiac failure	Normal
Yeast, auto. (alk.) B$_s$P [4]	7-13	241	204	(See footnote 4)	Sacrificed	Normal
Liver, desiccated	6-54	410	320	42 ? (40–90)	Cardiac failure	Normal
	6-62	327	239	42 ? (40–60)	Sacrificed	Normal
	6-79	318	230	41 ? (40–50)	Sacrificed	Normal

[1] R refers to riboflavin 0.12, N niacin 1.20, B$_s$ pyridoxine 0.20, Ch choline 10.00, P calcium pantothenate 0.50, I inositol 0.10, p para-aminobenzoic acid 0.1 mg. per kilo body weight daily.

[2] Animals in previous series (Wintrobe et al., '42 b; Follis et al., '43).

[3] Pig 7-11 was fed alkaline autoclaved yeast until the age of 138 days. Pig 7-40 was given 0.50 mg. calcium pantothenate per kilo body weight daily until the age of 103 days. Thus both animals received a larger supply of calcium pantothenate than they would have had, had the yeast supplement from the age of 3 weeks been dry autoclaved yeast. All pigs receiving yeast were fed 3 gm. per kilo body weight daily.

[4] The thiamine content of these yeast samples was not measured.

been no adequate justification for the assumption that the lack of thiamine was the specific cause of the changes observed. Surely classical beriberi, occurring as it does in persons consuming a diet consisting almost exclusively of polished rice, is a multiple deficiency disorder. The effects of therapy with thiamine in cases of "multiple neuritis" have not been such as to convince the critical observer that eventual improvement must necessarily have been attributed to thiamine (Brown, '41; Strauss, '43).

The only exceptions to the above statement are the reports of Williams et al., ('43) and Najjar and Holt ('43). Williams and coworkers

described symptoms and signs suggesting peripheral nerve degeneration in two experimental subjects given a diet lacking only in thiamine. Unfortunately no biopsy was made and thus it was impossible to state whether there was functional impairment only, or actual nerve degeneration as well. The same question arises in the case of the four subjects of Holt and Najjar's study in which "neuritis" developed. In view of the importance of thiamine in carbohydrate metabolism one must also consider the possibility that some of the symptoms and signs regarded as representing "polyneuropathy" may be the result of a metabolic disturbance in the muscles. Surely those signs which are promptly relieved by the administration of thiamine are the consequence of a metabolic defect rather than due to a histologic change.

It is of interest to compare our results with those of Van Etten, Ellis and Madsen ('40), also obtained with pigs. These workers observed neurological changes in animals fed autoclaved liver and whey as the source of the B vitamins, but found no such changes in pigs fed sodium sulfite, sulfur dioxide treated liver and whey. Autoclaving of liver for 6 hours at 20 pounds pressure, was found by our assays to reduce the pantothenic acid content from 105 to 34 μg. per gm.; autoclaving of whey in the same way, reduced the pantothenic acid from 33 to 4 μg. Incomplete data suggest that sulfite treatment of these substances as well as of yeast does not reduce their pantothenic acid content to the extent produced by autoclaving.

The requirement of the pig for pantothenic acid is at least 160 μg. per kg. body weight (Wintrobe, Follis, Alcayaga, Paulson and Humphreys, '43 b), and under certain conditions may be as high as 500 μg. (Ellis, Madsen and Miller, '43). The observations of Van Etten, Ellis and Madsen ('40) indicated that their autoclaved diet was deficient in more than one factor. In the light of our assays, pantothenic acid probably was one of these factors and the neurological changes observed by them were in all likelihood due, in part at least, to a lack of this vitamin. The absence of nerve degeneration in their animals which were fed sulfite treated diets, and in our pigs fed autoclaved yeast, can be explained by a failure to reduce the pantothenic acid content below the critical level.

SUMMARY

Additional experiments in pigs in which chronic thiamine deficiency was produced, have failed to support the claim that lack of thiamine causes degenerative changes in the nervous system.

It is pointed out that autoclaving destroys not only thiamine, but reduces the pantothenic acid content of yeast as well. It is suggested that certain effects heretofore attributed to lack of thiamine may have in reality been due to lack of pantothenic acid.

LITERATURE CITED

BESSEY, O. A. 1942 Personal communication.

BROWN, M. R. 1941 Alcoholic polyneuritis. J. Am. Med. Assn., vol. 116, p. 1615.

ELLIS, N. R., AND L. L. MADSEN 1944 The thiamine requirement of pigs as related to the fat content of the diet. J. Nutrition, vol. 27, p. 253.

ELLIS, N. R., L. L. MADSEN AND C. O. MILLER 1943 Pantothenic acid and pyridoxine as factors in the occurrence of locomotor incoordination in swine. (Abst.) J. An. Sci., vol. 2, p. 365.

FOLLIS, R. H., JR., M. H. MILLER, M. M. WINTROBE AND H. J. STEIN 1943 Development of myocardial necrosis and absence of nerve degeneration in thiamine deficiency in pigs. Am. J. Path., vol. 19, p. 341.

NAJJAR, V. A., AND L. E. HOLT, JR. 1943 The biosynthesis of thiamine in man. J. Am. Med. Assn., vol. 123, p. 683.

STRAUSS, M. B. 1943 Multiple neuritis. Differentiation of nutritional polyneuritis from other multiple neuritides. Res. Publ. Ass. Nerv. Ment. Dis., vol. 22, p. 141.

STRONG, F. M., R. E. FEENEY AND ANN EARLE 1941 Microbiological assay for pantothenic acid. Ind. Eng. Chem., Anal. Ed., vol. 13, p. 566.

SWANK, R. L. 1940 Avian thiamine deficiency. A correlation of the pathology and clinical behavior. J. Exper. Med., vol. 71, p. 683.

VAN ETTEN, C., N. R. ELLIS AND L. L. MADSEN 1940 Studies on the thiamine requirement of young swine. J. Nutrition, vol. 20, p. 607.

WILLIAMS, R. D., H. L. MASON, M. H. POWER AND R. M. WILDER 1943 Induced thiamine (vitamin B_1) deficiency in man. Arch. Int. Med., vol. 71, p. 38.

WINTROBE, M. M., R. ALCAYAGA, S. HUMPHREYS AND R. H. FOLLIS, JR. 1943a Electrocardiographic changes associated with thiamine deficiency in pigs. Bull. Johns Hopkins Hosp., vol. 73, p. 169.

WINTROBE, M. M., R. H. FOLLIS, JR., R. ALCAYAGA, M. PAULSON AND S. HUMPHREYS 1943b Pantothenic acid deficiency in swine. With particular reference to the effects on growth and on the alimentary tract. Bull. Johns Hopkins Hosp., vol. 73, p. 313.

WINTROBE, M. M., M. H. MILLER, R. H. FOLLIS, JR., H. J. STEIN, C. MUSHATT AND S. HUMPHREYS 1942a Sensory neuron degeneration in pigs. IV. Protection afforded by pantothenic acid and pyridoxine. J. Nutrition, vol. 24, p. 345.

WINTROBE, M. M., H. J. STEIN, M. H. MILLER, R. H. FOLLIS, JR., V. NAJJAR AND S. HUMPHREYS 1942b A study of thiamine deficiency in swine together with a comparison of methods of assay. Bull. Johns Hopkins Hosp., vol. 71, p. 141.

INEFFECTIVENESS OF VITAMIN E IN PREVENTING CHOLESTEROL DEPOSITION IN THE AORTA [1]

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(Received for publication May 16, 1944)

The fact that vitamin E deficiency causes an increase in the cholesterol content of muscles in rabbits, rats and chicks (Morgulis, et al., '38; Heinrich and Mattill, '43; Dam, ''44a) and affects at least part of the vascular system, i. e., the capillaries, in chicks (Dam and Glavind, '39) and in the rat's fetus (Mason, '42, '43) naturally leads to the question whether the presence or absence of vitamin E might not influence the deposition of cholesterol in the aorta of animals fed a diet rich in this substance.

An investigation of this question has been carried out with rabbits and chicks, species which are known to deposit cholesterol in the aorta during prolonged cholesterol-feeding. In the experiments with chicks the additional effect of orally administered lipocaic and inositol was studied, since these substances are known to exert an influence on the vascular symptoms in vitamin E deficient chicks (Dam and Glavind, '42); while lipocaic has also been reported by certain authors to counteract cholesterol atherosclerosis in rabbits, other investigators appear not to be in accord with this finding. The experiments with rabbits were carried out first. Since the aorta showed no significant changes it was decided in the chick experiments to extend the cholesterol determinations to certain other tissues in addition to the aorta.

EXPERIMENTAL

In the experiments with rabbits 3 groups of young animals weighing about 1400 gm. were reared on a basal diet of ground oats to which was added 5 mg. diacetate of 2-methyl-1, 4 napthohydroquinone per kg., supplemented with 50 gm. of carrots per animal per day. One group was given 1% powdered cholesterol mixed with the ground

[1] Aided by a grant from the Josiah Macy, Jr., Foundation.

oats [2] and another group received 100 mg. d, l-alpha-tocopherol acetate [3] per kg. of the oats-cholesterol mixture. After 85–97 days of feeding the animals were killed and the aortas, from the aortic valve to the bifurcation, were removed and analyzed for cholesterol after the method of Hoffmeyer and Bjørnsson ('40). This procedure involves hardening the tissue in 4% formaldehyde solution for 24 hours, removal of the adventitial fat by scraping, drying in vacuo at room temperature to constant weight, dissolution in 60% KOH by heating on a steam bath, extraction with ether, washing and evaporation to dryness. After dissolving the non-saponifiable matter in chloroform the cholesterol was determined in an aliquot part by the Liebermann-Burchard reaction. The results are presented in table 1.

Chicks were reared in ordinary brooders with wire screen bottom and fed, during the first 61 days of life, a basal diet deficient in vitamin E and containing 30% lard (diet 190, Dam, '44a). Supplements of cholesterol, tocopherol,[3] inositol and lipocaic [4] were given as indicated in table 2. The aortas of the chicks in each experimental group were pooled and analyzed for cholesterol by the same method used for rabbit aortas. In order to compare the possible effect on the aorta with that on other organs, livers, breast muscles and brains (cerebrum and cerebellum) were also examined, 1-gm. samples of these organs being dried in vacuo to constant weight without formalin treatment. For practical reasons it was necessary to pool the corresponding tissues from different individuals in some groups and therefore only average figures are presented (table 2). The experiments arranged between the horizontal lines in table 2 were carried out simultaneously.

RESULTS

Rabbit experiments

According to Hoffmeyer and Bjørnsson ('40) the upper limit for normal cholesterol values in rabbit aortas, as determined by their method, is 5 mg. per gm. dry weight. In the present experiment the group without ingested cholesterol showed values from 5.7 to 10.2 mg. per gm. The reason for this is not clear. The values for the aorta cholesterol in the two cholesterol-fed groups are irregular but increased values oc-

[2] Although cholesterol is usually fed as a solution in oil to facilitate its absorption, the relatively large amounts fed in the powdered form compensated for the lower absorbability and caused deposition in the aorta and other tissue.

[3] "Ephynal acetate," kindly supplied by Hoffmann-La Roche, Inc., Nutley, N. J.

[4] Kindly supplied by Dr. L. R. Dragstedt, University of Chicago and The Eli Lilly Research Laboratory.

cur not only in the group which received no added vitamin E but also in the group which received tocopherol. This latter substance, therefore, did not prevent the cholesterol deposition. It is of little importance for this conclusion that the basal diet was not devoid of vitamin E.

TABLE 1

Influence of feeding cholesterol and vitamin E on mortality and cholesterol content of the aorta of rabbits.

GROUP	ADDITION TO BASAL DIET	DAYS ON DIET UNTIL		INITIAL WEIGHT	FINAL WEIGHT	TOTAL CHOLESTEROL IN DRIED AORTA
		Death	Killed			
				gm.	*gm.*	*mg. per gm.*
		95		1430	927	5.7
		52		1550	1221	
			92	1347	1995	6.4
			91	1640	1498	6.5
1	none		92	1365	1623	9.5
			85	1355	1268	10.2
		37		1530	1122	
			92	1505	2175	9.7
		25		1425	940	
		50		1630	1144	
		93		1355	1476	10.6
		58		1422	1315	
		73		1375	1010	
2	1%		92	1335	1456	15.5
	cholesterol	25		1388	1080	
		48		1270	990	
		45		1415	1020	
		41		1461	879	
			88	1421	1244	32.7
		56		1316	1115	
	1%		97	1440	1380	5.1
	cholesterol		97	1345	1585	7.5
	+ 10 mg.%	56		1345	955	
3	*d, l*-alpha		92	1392	1458	18.7
	tocopherol		92	1345	1228	22.0
	acetate		92	1390	1510	58.6

While the rabbits grew slowly and irregularly on the basal diet and some of them died, the cholesterol feeding without added vitamin E caused a considerable increase in the mortality, making it necessary to include many more individuals in group 2 in order to obtain aortas which had been exposed to the cholesterol feeding for the same length of time as in the other groups. Ingestion of a substantial amount of d, l-alpha-tocopherol acetate lowered the mortality. This seems to in-

TABLE 2

Influence of cholesterol feeding with and without vitamin E, and with vitamin E and inositol or lipocaic, on the cholesterol content of the aorta and other organs in chicks during the first 61 days of life.

GROUP	ADDITION TO BASAL DIET	NO. OF ANIMALS		WEIGHT WHEN KILLED GM.	AORTA DRY WT.	CHOLESTEROL, AS MG. PER GM. OF TISSUE					
						Liver		Muscle		Brain	
		Initial	Final			Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.
1	none	9	4 [1]	368	8.54	3.22	11.1	1.45	5.80	15.5	75.0
2	10 mg.% *d, l*-alpha tocopherol acetate	8	8	422	9.43	3.55	11.2	0.88	3.35	15.9	74.2
3	2% cholesterol	10	6 [2]	390	27.5	50.9	151	1.95	7.96	15.9	75.2
4	as 3 + 10 mg.% *d, l*-alpha tocopherol acetate	10	10	365	28.6	46.9	138.5	1.04	4.10	15.0	72.8
5	same as 4	7	7	424	30.8	48.8	146	1.01	3.88		
6	as 4 + 1.5% inositol	7	6	344	29.7	30.8	95.2	1.19	4.64		
7	as 4 + 2% lipocaic	7	7	532	32.2	88.0	234	0.966	3.63		

[1] Four of the animals which died during the feeding had encephalomalacia and one had general exudative diathesis. Of the four surviving birds used for the determinations, two had a general exudative diathesis; and none had encephalomalacia.

[2] Three of the animals which died during the feeding had general exudative diathesis, none had encephalomalacia (2% cholesterol protects largely against the latter symptom). Four of the surviving birds had general exudative diathesis; none had encephalomalacia.

dicate that the basal diet did not furnish vitamin E in sufficient amount to enable the animals to resist a general deleterious effect of the cholesterol feeding. The life-prolonging effect of vitamin E in these experiments in which the basal diet is unbalanced, probably also as far as the protein is concerned, resembles the effect of this vitamin on rats reared on a diet with fatally low protein content (Dam, '44b).

The chick experiment

The chicks which were fed 2% cholesterol in addition to the high-fat diet had about three times as much cholesterol in the aorta as those which received the high-fat diet only. This finding that cholesterol-feeding causes deposition in the aorta in chicks is in accordance with those of Dauber and Katz ('42). The extent of cholesterol deposition was not significantly influenced by dietary supplements of vitamin E, or by additions of lipocaic or a large amount of inositol together with vitamin E. Since the diets contained yeast the experiments do not answer the question whether the cholesterol deposition would have been more marked if the basal diet had been completely free from inositol, but this circumstance does not interfere with the conclusion that vitamin E could not prevent the increase in aorta cholesterol even when lipocaic or inositol was added.

As already indicated, some workers (Huber et al., '37) have reported that lipocaic tends to inhibit deposition of cholesterol in the rabbit aorta while others (Vermeulen et al., '42) have been unable to confirm this. The present experiments, though carried out with another species, agree with the findings of Vermeulen et al., '42.

The data in table 2 show further that, just as was the case with respect to the aorta, the total cholesterol content of liver and brain was not influenced to any marked degree by the presence or absence of vitamin E, whereas the muscles showed a clear response. Additions of vitamin E to the basal diet, and to the basal diet with 2% cholesterol added, caused a decrease in muscle cholesterol somewhat greater than the increased deposition caused by the addition of cholesterol.

As was to be expected, the brain cholesterol did not change by cholesterol feeding whereas the liver took up a large quantity. The latter phenomenon was influenced in opposite directions by lipocaic and inositol. The finding that inositol reduced the cholesterol deposition in the liver is in agreement with the observations of Forbes ('43) but the finding that lipocaic increased it was surprising in view of what had been found in experiments with rabbits by Vermeulen et al. ('42).

Ingestion of high cholesterol together with high inositol seemed to have a noxious influence on the chicks since they had a mild perosis, were less vigorous and gained in weight more slowly than those receiving lipocaic or no addition to their cholesterol-rich diet. Ingestion of lipocaic together with cholesterol seemed to have an opposite effect. A more detailed study of the problems raised by these observations may throw some light on the effect of inositol and lipocaic on cholesterol metabolism.

SUMMARY

1. Vitamin E, fed as 10 mg. % d, l-alpha-tocopherol acetate in the basal diet, failed to modify the deposition of cholesterol in the aorta in (a) rabbits fed a diet of ground oats and carrots plus 1% cholesterol, and in (b) chicks fed an artificial diet deficient in vitamin E and containing 30% lard and 2% cholesterol, with or without addition of 1.5% inositol or 2% lipocaic.

2. The vitamin E supplement prevented a high mortality occurring in rabbits fed the oats-carrots-cholesterol diet, reduced the normal cholesterol content of muscles in chicks fed the basal artificial diet, and the increased muscle cholesterol of chicks fed the same diet supplemented with 2% cholesterol; the explanation of these effects is obscure.

3. In chicks inositol feeding reduced, and lipocaic feeding increased, the extensive deposition of liver cholesterol following additions of the latter to the basal diet.

LITERATURE CITED

DAM, H. 1944a Studies on vitamin E deficiency in chicks. J. Nutrition, vol. 27, p. 193.

———— 1944b Vitamin E and length of life of rats fed a diet with fatally low protein content. Proc. Soc. Exp. Biol. and Med., vol. 55, p. 55.

DAM, H., AND J. GLAVIND 1939 Alimentary exudative diathesis a consequence of E-avitaminosis. Nature, vol. 143, p. 810.

———— 1942 Factors influencing capillary permeability in the vitamin E deficient chick. Science, vol. 96, p. 235.

DAUBER, D. V., AND L. N. KATZ 1942 Experimental cholesterol atheromatoses in an omnivorous animal, the chick. Arch. Pathol., vol. 34, p. 937.

FORBES, J. C. 1943 Lipotropic action of inositol. Proc. Soc. Exp. Biol. and Med., vol. 54, p. 89.

HEINRICH, M. R., AND H. A. MATTILL 1943 Lipids of muscle and brain in rats deprived of tocopherol. Proc. Soc. Exp. Biol. and Med., vol. 52, p. 344.

HOFFMEYER, J., AND J. BJØRNSSON 1940 Quantitative chemical determination of experimentally produced aortic lipoidosis in rabbits. Acta Pathologica et Microbiologica Scandinavica, vol. 17, p. 46.

HUBER, M. J., G. O. BROUN AND A. E. CASEY 1937 Prevention of cholesterol arteriosclerosis in the rabbit by use of pancreatic extract (lipocaic). Proc. Soc. Exp. Biol. and Med., vol. 37, p. 441.

MASON, K. E. 1942 Changing concepts of the antisterility vitamin (vitamin E). Yale J. Biol. and Med., vol. 14, p. 605.

———— 1943 A hemorrhagic state in the vitamin E-deficient fetus of the rat. Essays in Biology (in honor of H. M. Evans). Univ. Calif. Press, p. 401.

MORGULIS, S., V. M. WILDER, H. C. SPENCER AND S. H. EPPSTEIN 1938 Studies on the lipid content of normal and dystrophic rabbits. J. Biol. Chem., vol. 124, p. 755.

VERMEULEN, C. L., L. R. DRAGSTEDT, D. E. CLARK, O. C. JULIAN AND J. G. ALLEN 1942 Effect of the administration of lipocaic and cholesterol in rabbits. Arch. Surgery, vol. 44, p. 260.

TOXICITY OF FRACTIONS OF HOG LIVER FATTY ACIDS TO CHICKS FED A VITAMIN E DEFICIENT DIET [1]

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TWO FIGURES

(Received for publication May 16, 1944)

It is known that chicks reared on a vitamin E deficient diet will develop encephalomalacia or exudative diathesis when the diet contains a sufficient amount of fat containing highly unsaturated fatty acids. Which of the two symptoms will be the main clinical manifestation of the disorder is largely determined by the composition of the diet (Dam, '44). The nature and amount of the fatty acids in the diet is particularly influential in this respect. For instance, with the basal diet 182 (Dam, '44) the main symptom will be exudative diathesis when the fat supplement of the diet (about 5%) consists of the fatty acid mixture from cod liver oil, but encephalomalacia when it consists of the fatty acids from hog liver fat.

The various highly unsaturated fatty acids are, at present, not easily available in sufficient quantities for a comparison of their ability to provoke these two symptoms. It has therefore been attempted, as a preliminary step in this investigation, to divide the fatty acids from hog liver fat into three fractions of varying degree of unsaturation and to compare the effect of these fractions on the symptoms.

EXPERIMENTAL

Seven and one-half kilograms of powdered dried hog liver were extracted with ether in a continuous extractor for 24 hours. The fat was saponified at room temperature in ether solution with 100% excess of potassium hydroxide in methanol for 24 hours, after which water was added and the non-saponifiable fraction removed by many shakings with ether. After acidification with HCl, the fatty acids were extracted with ether, washed with water, dried with sodium sulfate and the ether removed in vacuo. The fractionation of the fatty acids was carried out

[1] Aided by a grant from the Josiah Macy, Jr., Foundation.

in accordance with the method of Brown and Stoner ('37); namely, by preparing a 10% solution in acetone, freezing at three different temperatures, filtering and washing. The following fractions were obtained: fraction 1, separated from the acetone solution above — 20°C., solid at room temperature, iodine value 5, amount 241 gm.; fraction 2, separated between — 20°C. and — 72°C., liquid at room temperature, solid at 0°C., iodine value 104, amount 216 gm.; fraction 3, remaining in solution at —72°C., liquid at 0°C., iodine value 242, amount 200 gm.

The three fractions were tested with groups of ten newly hatched chicks as described by Dam ('44), first as 4% supplements to the vitamin E-free diet 182 (without cod liver oil). No other fat was given other than 1 drop of oleic acid (29 mg.) per animal twice a week as solvent for the concentrates of vitamins A and D. The fractions were added fresh to the diet every day, the bulk of them being kept in vacuo at 0°C. during the period of the experiment. The peroxide value of the most unsaturated fraction, determined after the method of King et al. ('33), was less than 4 milliequivalents per 100 gm. at the end of the experiment and the iodine was unchanged. In the case of fraction 3 which proved the most active one, the protective action of d, l-alpha-tocopherol acetate was tested, and also the effect of a much smaller amount of this fraction was determined, namely, 1 drop (24 mg.) per animal per day during the first 10 days, the daily dose being increased by 1 drop per animal for every subsequent 10-day period. The amount given in this way corresponded to approximately 0.5–0.6% of the diet consumed.

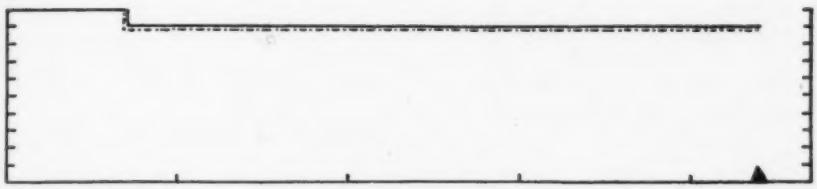
The method of observing and recording the symptoms was as previously described (Dam, '44). The graphs give a survey of the results.

RESULTS

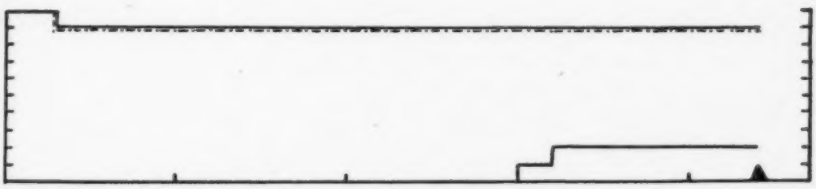
The least unsaturated fraction 1 (exp. 2) gave only two instances of exudate, both fully developed but appearing rather late, and no encephalomalacia. Since neither oleic acid (Dam, '43, '44), palmitic, nor stearic acids (unpubished findings) give rise to symptoms when fed in similar amounts, the exudates must in this case be attributed to the small amount of highly unsaturated acids which accompanied the saturated acids in this fraction.

Fraction 2, consisting mainly of oleic acid together with small amounts of more highly unsaturated acids such as linoleic, produced both exudates and encephalomalacia with about the same frequency (exp. 3), the exudates appearing somewhat earlier than in experiment 2. One bird had an intrapericardial exudate, as previously observed (Dam, '44) in experiments with hog liver fatty acids.

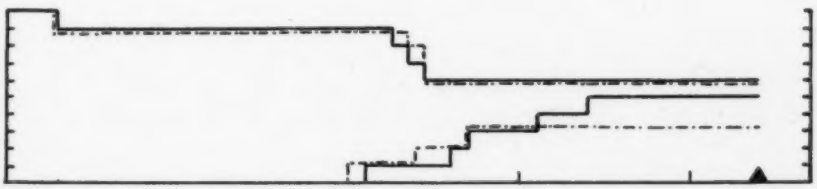
Fraction 3, consisting mainly of linoleic and more unsaturated acids, gave a very early onset of severe encephalomalacia which killed nearly all the animals between the tenth and the twenty-first days of the experiment (exp. 4). At autopsy some of the birds in this group showed redness (fine hemorrhage) of the subcutaneous adipose tissue, which is the first stage in the development of exudate and is arbitrarily counted as a "half exudate" in the graph. The deleterious effect of



< 10 Days >
Exp. 1. Diet 182 without cod liver oil



Exp. 2. Diet as in Exp. 1 + 4% of fraction 1

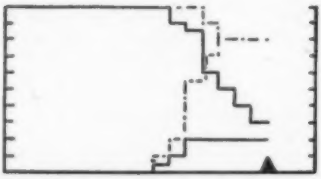


Exp. 3. Diet as in Exp. 1 + 4% of fraction 2

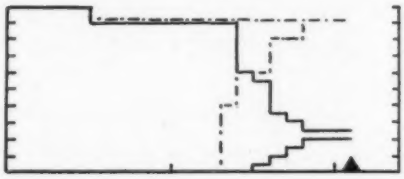
Abscissa: days.
Ordinate: number of animals.
Full line from bottom: development of exudate.
Full line from top: deaths of animals without exudate.
Broken line from bottom: development of encephalomalacia.
Broken line from top: deaths of animals without encephalomalacia.

fraction 3 was completely prevented when the diet contained 10 mg. % d, l-alpha-tocopherol acetate [2] (exp. 5).

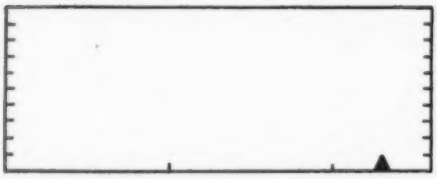
When fraction 3 was given in relatively small amounts corresponding to 0.5–0.6% of the diet (exp. 6), both encephalomalacia and fully de-



Exp. 4 Diet as in Exp. 1+4% of fraction 3



< 10 Days >
Repetition of Exp. 4



Exp. 5 Diet as in Exp. 4 +10 mg. % d, l-alphatocopherol acetate.

Exp. 6. Diet as in Exp. 1 + small amount of fraction 3 (compare text)

Abscissa: days.

Ordinate: number of animals.
Full line from bottom: development of exudate.
Full line from top: deaths of animals without exudate.
Broken line from bottom: development of encephalomalacia.
Broken line from top: deaths of animals without encephalomalacia.

[2] Ten mg. % is probably far above the limit of the protective dose. This amount was chosen because it has been used with many other diets and always gave full protection. With diet 182 (containing 5% cod liver oil) the limit for protection has been found to lie between 0.625 and 1.25 mg. % in a 5-week experiment.

veloped exudates occurred, but these symptoms appeared more slowly than when the fraction was fed as 4% of the diet. Encephalomalacia was still the predominant symptom though less so than when 4% was given. None of the fractions tested gave encephalomalacia as the only symptom. It would nevertheless be of interest to study the effect of the individually fatty acids, especially those occurring in fraction 3.

DISCUSSION

The following represents a tentative interpretation of the observations reported here. As shown in previous experiments (Dam, '44) the time which elapses from the beginning of feeding to the onset of symptoms is sufficient for the accumulation of dietary fatty acids in the tissue lipids to such an extent that it influences the iodine value of the phospholipids, those of brain tissue being less altered than those of muscle and liver. It seems likely, therefore, that the presence of a certain amount of highly unsaturated fatty acids in the tissue lipids (phospholipids or others) is a prerequisite to the development of the symptoms. Since the two symptoms are both dependent upon ingestion of highly unsaturated fatty acids and preferably affect tissue which is rich in lipids (adipose tissue, brain tissue), and since the first gross change in the tissues in both cases consists of fine hemorrhages followed by edema or exudation, exudative diathesis and encephalomalacia may be due to the same biochemical disturbance occurring in different tissues — a possibility which has been referred to earlier (Dam, '44). It is known that the lipids of brain tissue are influenced less by the dietary fat than is the fat of adipose tissue, and it is therefore possible that ingestion of a small amount of unsaturated acids will mainly affect the adipose tissue or muscle tissue and cause exudative diathesis in this tissue when given sufficient time (exp. 2). However, if the brain tissue is more sensitive to a certain change of the lipids, the ingestion of a large amount of unsaturated acids may cause severe cerebellar or cerebral disturbances leading to death before the other symptom is fully developed (exp. 4).

The way in which the cellular damage is brought about could be one of the following: (1) The damage is caused by some abnormal oxidation of the highly unsaturated fatty acids in the tissue which can be prevented by the presence of a sufficient quantity of vitamin E, and can, locally, cause the destruction of the vitamin if the latter is present in such minute quantity that it cannot halt the oxidation, or (2) the presence of highly unsaturated fatty acids imparts such physical or physico-chemical properties to the lipid phase of the vascular wall, that

the latter, latently damaged by the absence of vitamin E, is more apt to give way to hydrostatic and osmotic forces. The possibility that destruction of traces of vitamin E in the diet through rancidity might play a role in the development of the symptoms has already been ruled out (Dam, '44).

The above-mentioned considerations do not explain the previously observed modifying effect of the protein-carbohydrate ratio, lipocaic, inositol or cholesterol; neither do they explain the quantitative difference in the effect of hog liver fat and cod liver oil on the two symptoms.

SUMMARY

Three fractions of hog liver fatty acids were tested for their effectiveness in producing exudative diathesis and encephalomalacia in chicks reared on a vitamin E deficient diet.

When fed as 4% supplements to the diet, the least unsaturated fraction (iodine value 5) had little effect and gave only exudates; the intermediate fraction (iodine value 104) gave both exudates and encephalomalacia, whereas the most unsaturated fraction (iodine value 241) caused a rapid onset of severe encephalomalacia and early death of the animals. The deleterious effect of this fraction could be prevented by d, l-alpha-tocopherol acetate. When fed in smaller amounts corresponding to 0.5–0.6% of the diet, the most unsaturated fraction gave both exudates and encephalomalacia.

The significance of the incorporation of dietary fatty acids in tissue lipids as a prerequisite to the symptoms is discussed.

ACKNOWLEDGMENT

Thanks are due to F. Hoffmann-La Roche, Inc., Nutley, N. J., for d, l-alpha-tocopherol acetate (Ephynal acetate, Roche) and to Armour and Company, Chicago, for powdered dried hog liver.

LITERATURE CITED

BROWN, J. B., AND G. STONER 1937 Studies on the chemistry of the fatty acids. I. The purification of linoleic acid by crystallization methods. J. Am. Chem. Soc., vol. 59, p. 3.

DAM, H. 1943 Effect of cod liver oil and rancidity on certain vitamin E deficiency symptoms. Proc. Soc. Exp. Biol. and Med., vol. 52, p. 285.

———— 1944 Studies on vitamin E deficiency in chicks. J. Nutrition, vol. 27, p. 193.

KING, A. E., H. L. ROSCHEN AND W. H. IRWIN 1933 An accelerated stability test using the peroxide value as an index. Oil and Soap, vol. 10, p. 105.

MEAD JOHNSON AND COMPANY 'B-COMPLEX' AWARD

Nominations are solicited for the 1945 Award of $1,000 established by Mead Johnson and Company to promote researches dealing with the B complex vitamins. The recipient of this Award will be chosen by a Committee of Judges of the American Institute of Nutrition and the formal presentation will be made at the annual meeting of the Institute at Cleveland on May 8, 1945.

The Award will be given to the laboratory (non-clinical) or clinical research worker in the United States or Canada who, in the opinion of the judges, has published during the previous calendar year January 1st to December 31st the most meritorious scientific report dealing with the field of the 'B-complex' vitamins. While the award will be given primarily for publication of specific papers, the judges are given considerable latitude in the exercise of their function. If in their judgment circumstances and justice so dictate, it may be recommended that the prize be divided between two or more persons. It may also be recommended that the award be made to a worker for valuable contributions over an extended period but not necessarily representative of a given year. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award.

To be considered by the Committee of Judges, nominations for this award for work published in 1944 must be in the hands of the Secretary by January 15th, 1945. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Committee of Judges in its consideration of the nomination.

ARTHUR H. SMITH
Wayne University College of Medicine
Detroit, Michigan

SECRETARY, AMERICAN INSTITUTE OF NUTRITION

BORDEN AWARD IN NUTRITION

The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. The award will be made primarily for the publication of specific papers, but the judges may recommend that it be given for important contributions over an extended period of time. The award may be divided between two or more investigators. Employees of the Borden Company are not eligible for this honor.

The formal presentation will be made at the annual meeting of the Institute at Cleveland, May 8, 1945. To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee by January 15, 1945. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award.

FREDERICK J. STARE
Harvard Medical School
Boston, Massachusetts

CHAIRMAN, NOMINATING COMMITTEE